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Original Submission

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February 23, 2010

Dr. Robert Martin, Ph.D.
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: PolyGlycopleX® (PGX®) GRAS Notification

Dear Dr. Martin:

In accordance with proposed 21 CFR § 170.36 (a notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the representative of the notifier, Inovo Biologic Inc., 104 - 1240 Kensington Rd. NW, Suite 409, Calgary, Alberta Y2N4Y7, Canada, a GRAS notification for the use of PolyGlycopleX® (PGX®) as a source of fiber in the diet in conventional and medical foods at a maximum consumption level of 13,070 mg/day. A GRAS expert panel dossier, setting forth the basis for the GRAS determination, as well as *curriculum vitae* of the members of the GRAS panel, are enclosed.

Best regards,

(b) (6)

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

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1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36 (c) (1)

PolyGlycopleX® (PGX®) has been determined to be generally recognized as safe (GRAS) and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

_____ Date 23 February 2010

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
801 N. Orange Avenue
Suite 710
Orlando, FL 32801

(i) Name and Address of the Notifier

Inovo Biologic Inc.
104 - 1240 Kensington Rd. NW, Suite 409
Calgary, Alberta
Y2N4Y7, Canada

Agent of the Notifier:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
801 N. Orange Ave. Suite 710
Orlando, FL 32801
Telephone: 407-802-1400
Facsimile: 407-802-1405
Email: gburdock@burdockgroup.com

(ii) Common Name of the Notified Substance

The common name of PolyGlycopleX®, for the purposes of this GRAS Notification has been defined as:

PolyGlycopleX®, a soluble polysaccharide fiber complex

(iii) Conditions of Use

PGX® may be used as an ingredient in the food groups shown in Table 1 in order to provide a source of fiber, at levels up to 13,070 mg *per* day, by individuals who desire additional fiber in their diet. In addition, an amendment to the GRAS determination (dated October 15, 2009, attached) outlined the determination that PGX® is GRAS for use in medical foods at an aggregate daily intake of up to 13,070 mg/day, as monitored by a health care provider.

Table 1. Food groups selected for PGX® supplementation*

Food Category	Intended use level (ppm)
Yogurts	11,110
Milk shakes and fruit smoothie-type drinks	10,420
Frozen yogurt, ice cream bar, and puddings	6,670
White and whole wheat breads	50,000
Cookies	83,330
Breakfast bars	62,500
Granola-type bars	62,500
Noodles	17,860
Whole wheat cereals	83,330
Lasagna and macaroni/cheese	10,000
Fruit juices and fruit juice bars	10,420
Cereal beverage	10,420

*The food categories correspond to those listed in 21 CFR 170.3(n).
ppm = parts *per* million

(iv) Basis of GRAS Determination

Pursuant to 21 CFR § 170.3, the use of PGX® as an ingredient in food categories shown in Table 1 at an intended maximum 90th percentile consumption of 13,070 mg *per* day, has been determined GRAS by scientific procedures for its intended conditions of use. The safety of PGX® for this use is supported by acute, subchronic, chronic, carcinogenesis, reproductive, and genotoxicity studies, as well as clinical trials on either PGX® or its components. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food.

(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for Food and Drug Administration's (FDA) review and copying at a reasonable time at the office of:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
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Alternatively, copies of data and information can be provided to FDA upon request, by contacting Dr. Burdock.

2. Detailed Information about the Identity of the Notified Substance

A. Identity

PGX® is an off-white granular powder composed of an agglomeration of the food-grade, water-soluble polysaccharides: konjac powder[1], sodium alginate, and xanthan gum. The general descriptive characteristics of PGX® are presented in Table 2. The chemical name of PGX is: (α-D-glucurono-α-D-manno-β-D-manno-β-D-gluco),(α-L gulurono-β-D mannurono), β-D-gluco-β-D-mannan.

The three water-soluble polysaccharides are processed through a patented EnviroSimplex® manufacturing process that results in a soluble polysaccharide product with unique viscosity, rheological, and sedimentation properties. Visually, the manufacturing process results in a granular product that is easily dissolved in water, while a simple blend of the three raw ingredients results in a flour-like powder that produces a clumpy, paste-like consistency when added to water (Figure 1).

[1] Konjac powder is also known as konjac flour.

Table 2. General description of PGX®

Appearance	Off-white granular powder
Packaging	Tight Container
Storage	25°C
Stability	2 years
Labeling	PolyGlycopleX®, PGX®
Functionality in Food	Source of fiber

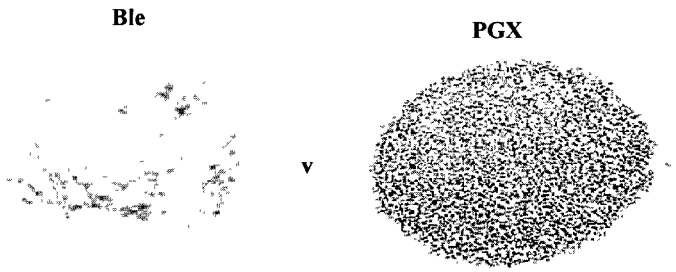

Figure 1. Visual differences between raw ingredient blend *vs.* PGX®

PGX® is relatively slow in its rate of increasing viscosity, when compared to the three individual components. Alginate does not increase in viscosity over time, while konjac and xanthan gum initially have a faster rate of viscosity (*i.e.*, the first 30 minutes of viscosity formation), compared to PGX® (Figure 2). However, between 30 and 120 minutes PGX increases in viscosity to greater than an additive effect of the three raw ingredients. This increased viscosity indicates that the three raw materials are interacting to form a new product with different viscosity characteristics.

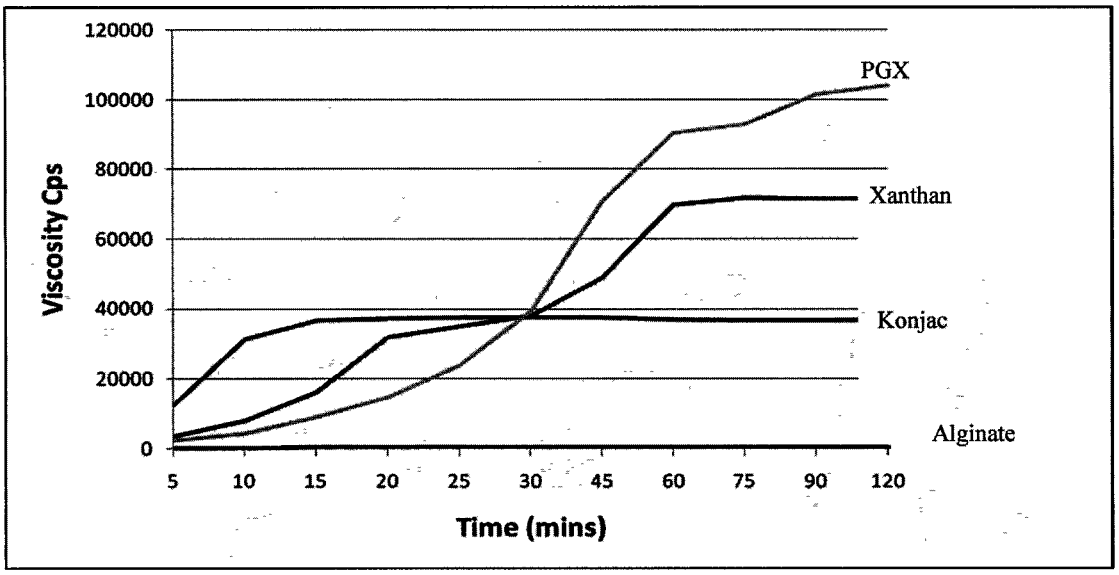

Figure 2. Comparison of the viscosity of PGX® to the raw ingredients used in the manufacture of PGX®.

Interactions between binary mixtures of polysaccharides are well known and have been extensively investigated by mechanical spectroscopy (*i.e.*, rheology). Dea *et al.* (1972)[2] postulated that a binary interaction occurs between various polysaccharides, as exemplified by a proposed interaction between xanthan gum and konjac visualized in Figure 3. PGX® was the subject of a recent published manuscript describing the ternary interactions that occur during the production of PGX®, forming a unique substance.[3] To evaluate the potential for the interaction between the components of PGX®, viscosity measurements were made on a range of ternary mixtures at a fixed concentration of 0.5% with increasing molar ratios of the sodium alginate fraction. The results indicated that the sodium alginate was exerting an additional effect when the mixtures had been subjected to the EnviroSimplex® manufacturing process).

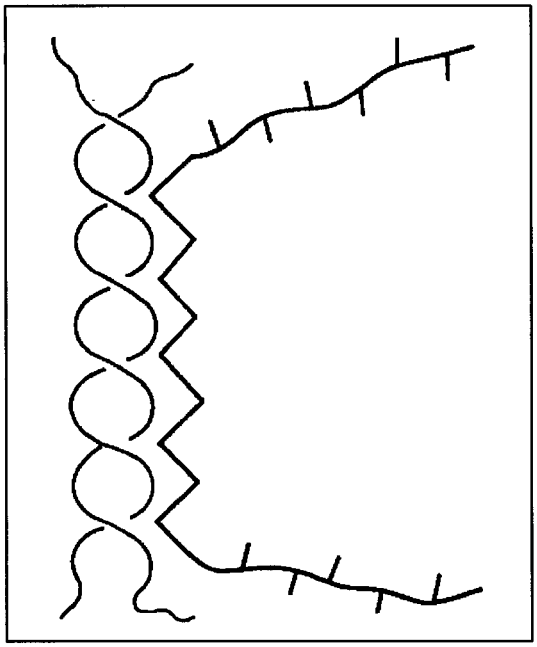

Figure 3. Postulated binary interaction between xanthan and konjac gums (Dea *et al.*, 1972)

The rheological observations were supported by sedimentation velocity measurements conducted with an analytical ultracentrifuge. The determination of sedimentation coefficient distribution plots is one method to characterize molecular integrity and to differentiate between different polysaccharide substances. Under the conditions of the study, the apparent sedimentation coefficient$_{S20,w}$ distributions for konjac glucomannan, alginate and xanthan were ~1.6S, ~1.3S and ~3.5S.[4] Sedimentation coefficient distribution plots were then generated for the ternary mixtures of the unprocessed raw ingredients when mixed (TM1, Figure 4 and Figure 5) and PGX® that has resulted from the patented manufacturing process (Figure 6 and Figure 7). For both samples, significant amounts of higher sedimenting material were observed up to an ionic strength of 0.01M. At higher levels, the appearance of sedimenting material was suppressed

[2] Dea, I.C.M., McKinnon, A.A., and Rees, D.A. (1972) Tertiary and quarternary structure in aqueous polysaccharide systems which model cell wall cohesion. Reversible changes in conformation and association of agarose, carrageenan, and galactomannans. *Journal of Molecular Biology*; 68: 153-172.

[3] Abdelhameed, A.S., Ang, S., Morris, G.A., Smith, I., Lawson, C., Gahler, R., Wood, S., and Harding, S.E. (2010) An analytical ultracentrifuge study on ternary mixtures of konjac glucomannan supplemented with sodium alginate and xanthan gum. *Carbohydrate Polymers*; DOI: 10.1016/j.carbpol.2010.01.043.

[4] 1S = 10^{-13} sedimentation coefficients (s)

(Figure 8 and Figure 9). A comparison of these two figures indicates that the PGX® behaves differently from a simple mixture of the three components.

The results from this technique (which avoids any complications from separation or column media by being a pure "free solution" technique) show that through the unique manufacturing process the complexing of konjac, xanthan an alginate show a significant interaction. Further, this work shows that a molecular weight shift to a higher level occurs when the alginate is present in the mixture, which is consistent with the formation of a complex.

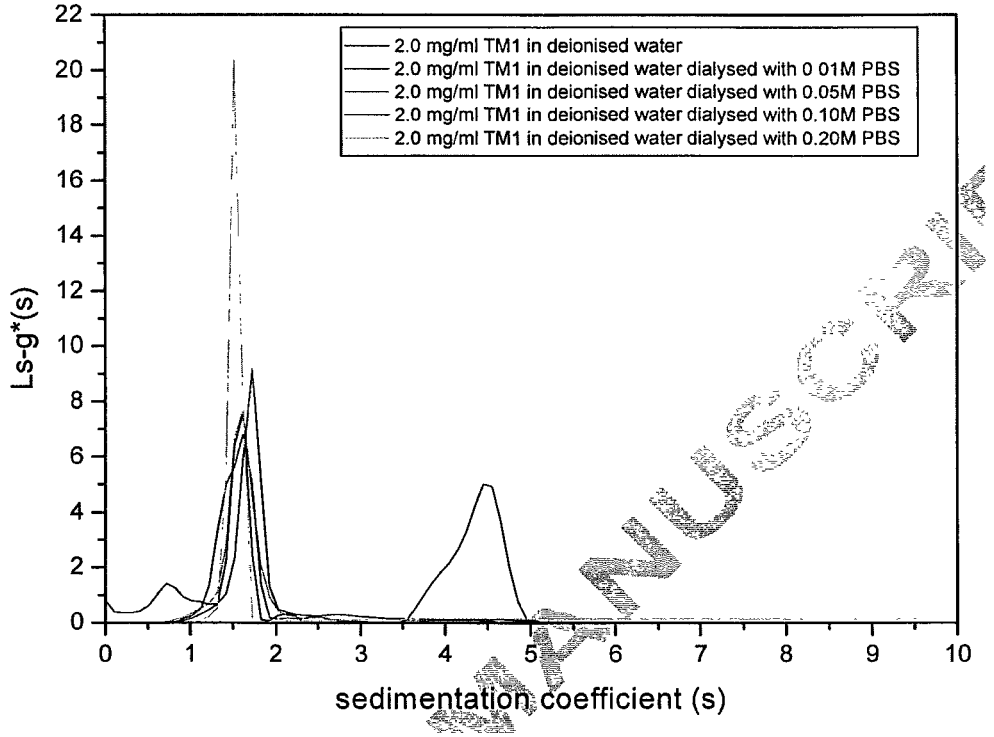

Figure 4. Apparent sedimentation concentration distributions for TM1 at ionic strengths 0 - 0.01M

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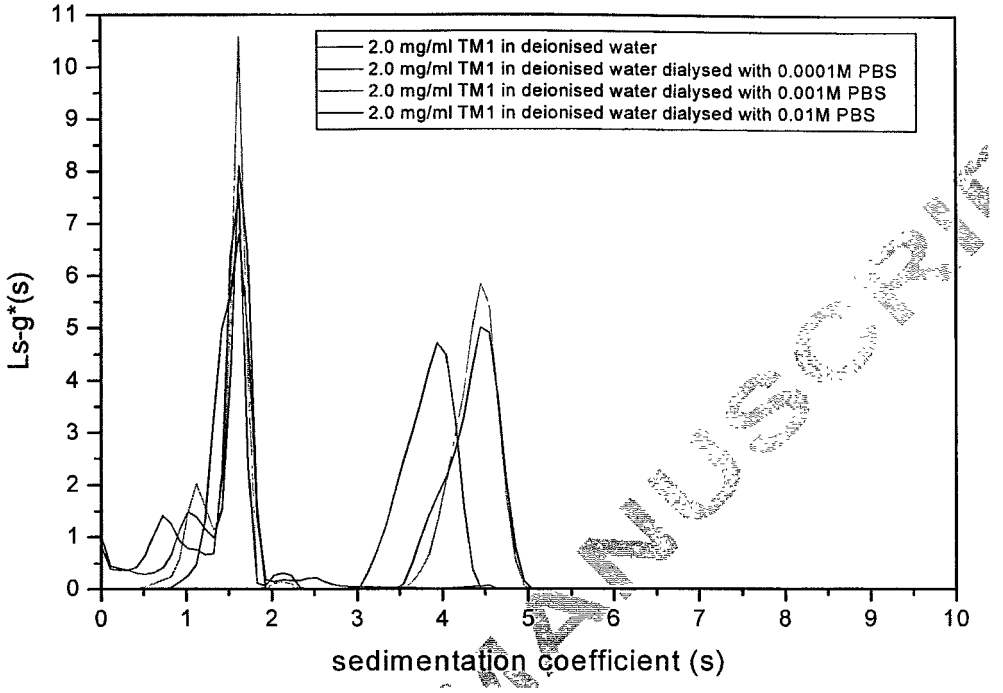

Figure 5. Apparent sedimentation concentration distributions for TM1 at ionic strengths 0 - 0.2M

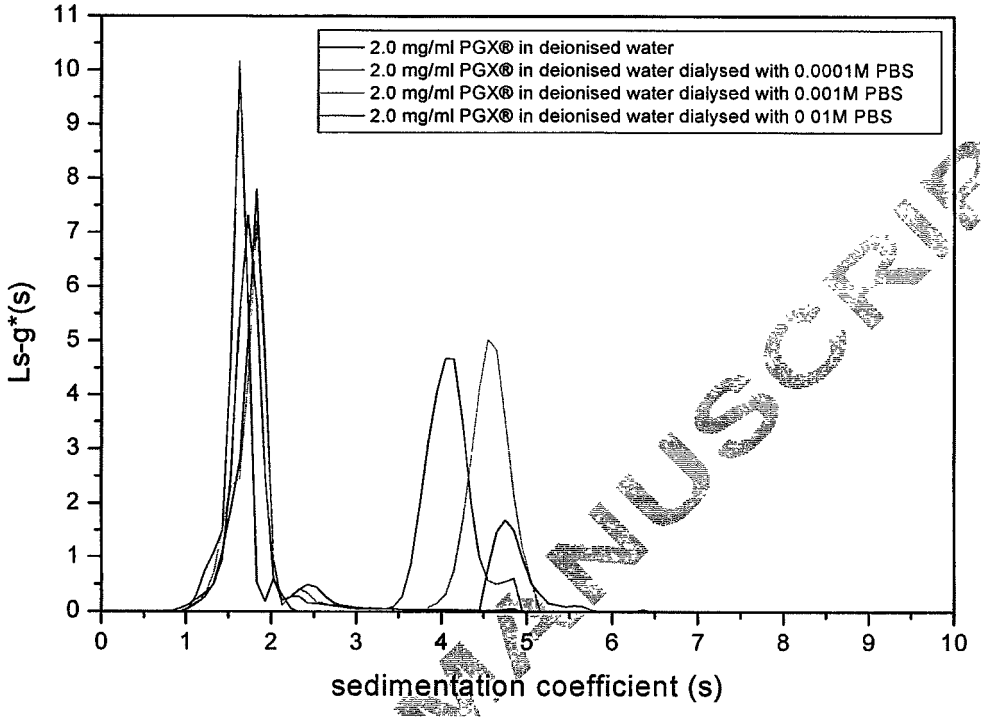

Figure 6. Apparent sedimentation concentration distributions for PGX® at ionic strengths 0 - 0.01M.

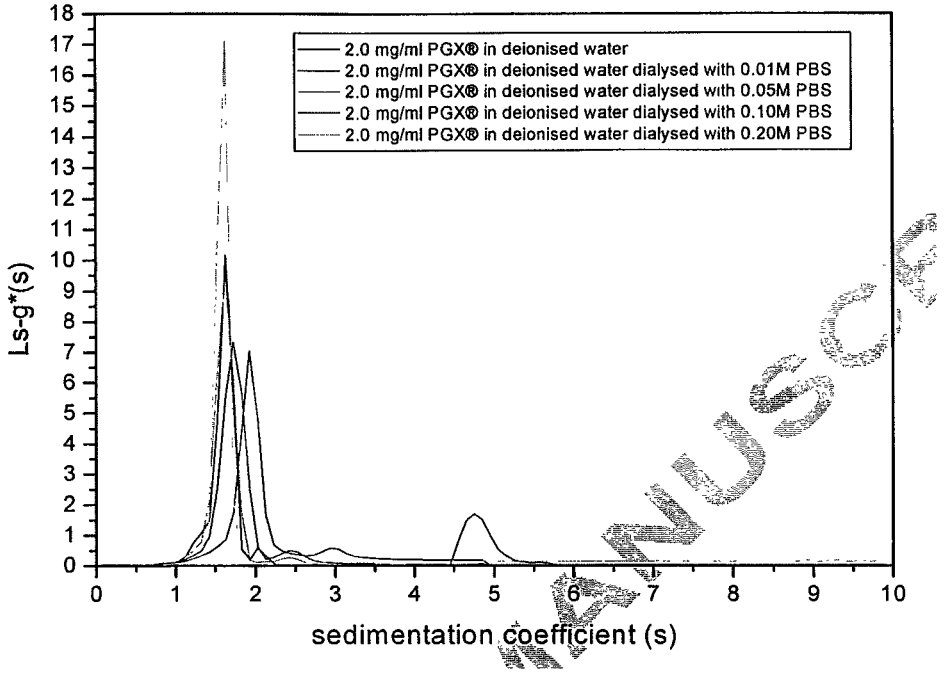

Figure 7. Apparent sedimentation concentration distributions for PGX® at ionic strengths 0 - 0.2M

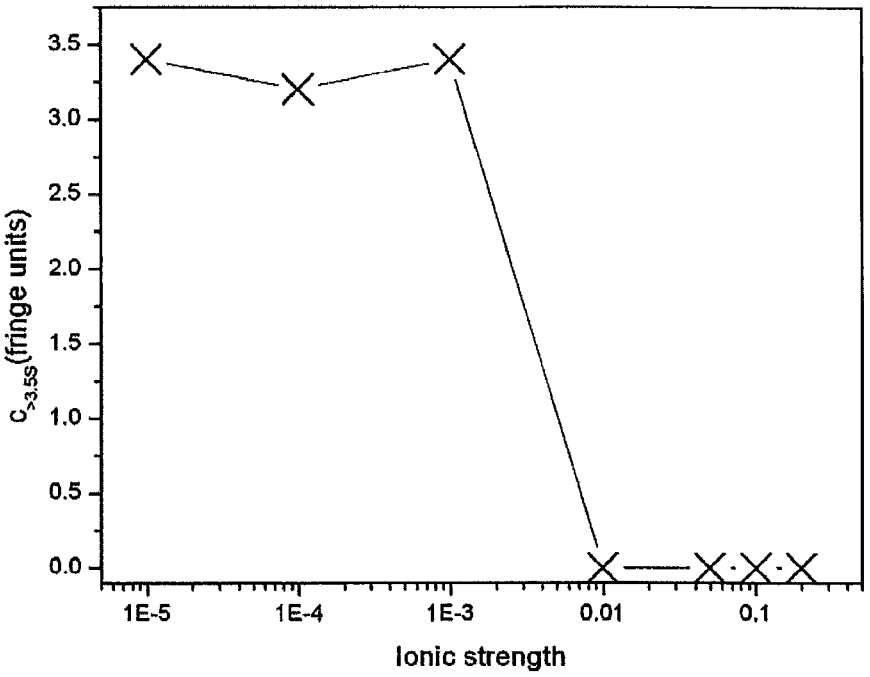

Figure 8. Effect of ionic strength (expressed in molar units) on the amount of material with a sedimentation coefficient >3.5S for TM1

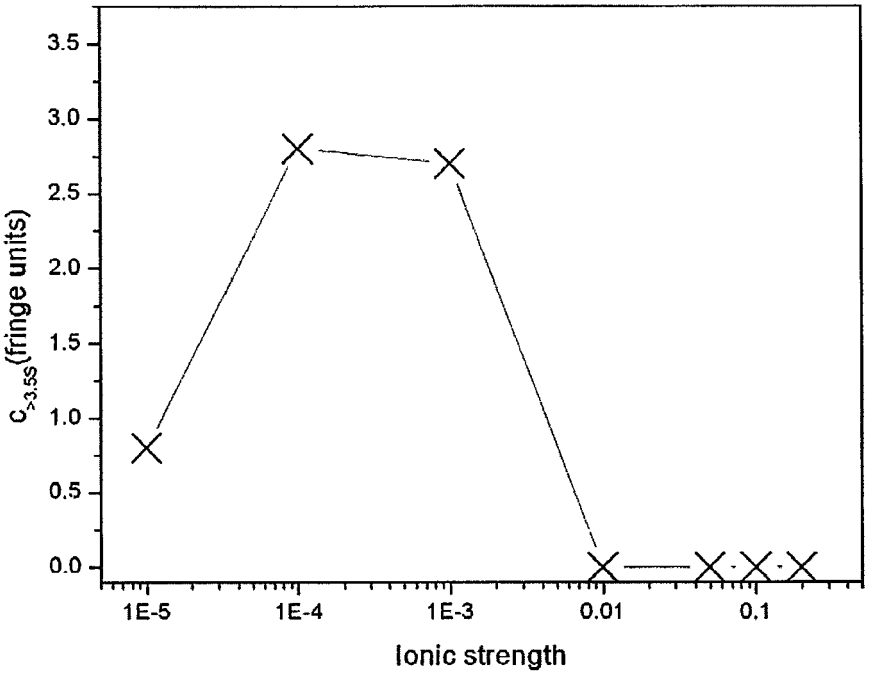

Figure 9. Effect of ionic strength (expressed in molar units) on the amount of material with a sedimentation coefficient >3.5S for PGX®

In addition to the above studies, an additional study on the precipitation of alginate out from the PGX® ingredient was conducted. Previous studies have shown that the alginate salt can be precipitated out of a simple solution with the addition of calcium ions.[5] The study was conducted in which the amount of PGX® in solution was compared to the equivalent amount of sodium alginate that was utilized in the production of PGX®. Treatment of PGX® in solution (0.5 – 0.05% in water) with calcium failed to produce any precipitate, whereas solutions of pure sodium alginate at concentrations comparable to those found in PGX® (0.075 – 0.0075% in water) produced a clearly observed precipitation (Figure 10). This finding is clearly consistent with the hypothesis that the sodium alginate in PGX® is unavailable to calcium ions due to complex formation with in the new polysaccharide matrix.

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[5] FAO Compendium of Food Additive Specifications. Sodium alginate; http://www.fao.org/docrep/W6355E/w6355e0x.htm#TopOfPage; site visited February 18, 2010

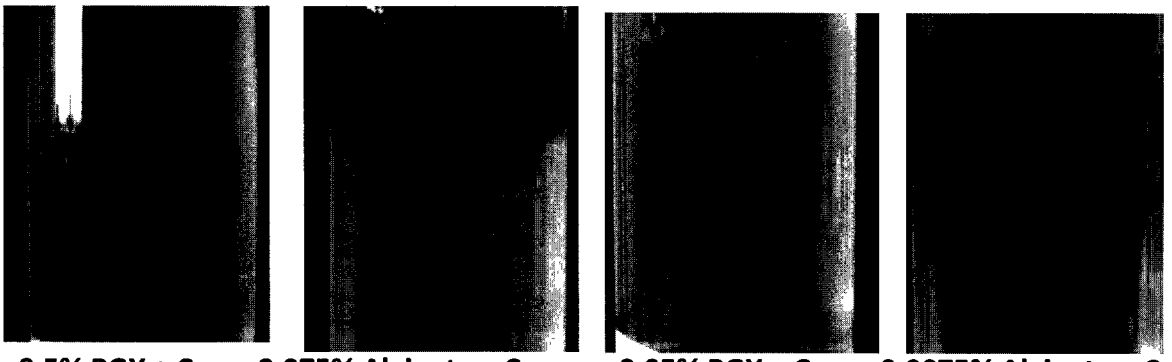

Figure 10. The effect of calcium treatment on PGX® and sodium alginate

Only under relatively harsh treatment *via* methylation or hydrolysis conditions and evaluation with ^{1}H-nuclear magnetic resonance spectroscopy of these partially degraded polysaccharides and the resulting monosaccharides was it possible to determine that PGX® is composed of the monosaccharides contained in the raw components (konjac, xanthan and alginate) (data not shown).

In summary, physical analysis of PGX® *via* rheology, ultracentrifugation and precipitation techniques resulted in the conclusion that PGX® is a novel entity. The rheology of PGX® is significantly higher than those of the individual components, conclusively showing interactions at the polymer level. The ultracentrifugation study shows a significant shift to a higher molecular weight, consistent with the formation of a unique complex, and the precipitation study confirms that the alginate fraction is complexed and unavailable for precipitation. Overall, the studies indicate that an interaction is occurring between the raw components at the binary and ternary levels such that a novel ingredient is formed.

Common or Usual Name:

The common name of PolyGlycopleX® has been defined as:

PolyGlycopleX®, a soluble polysaccharide fiber complex.

B. Composition

The chemical composition of PGX® is summarized in Table 3. PGX® is a mixture of carbohydrates, fiber, ash, protein and fat.

Table 3. Typical analysis of the major components of PGX®

	Batch Analysis Results (n=5)	
Analysis	Range	Average
Carbohydrates (%)	80 - 85.4	83.6
Total dietary fiber (%)	82.4 - 91.6	84.74
Ash (total) (%)	5 – 6.2	5.72
Protein (%)	1.8 – 2.2	2.04
Fat (%)	<0.1 – 0.1	<0.1

C. Method of Manufacture of PGX®

PGX® is manufactured by initially combining konjac powder, sodium alginate and xanthan gum, then a patented manufacturing processing forms an interlocking matrix of the individual components, which undergo agglomeration and drying to form the final novel product (Figure 11).

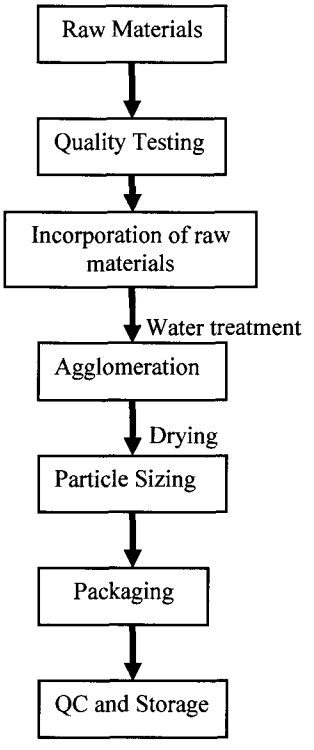

Figure 11. Schematic of the PGX® manufacturing process

D. Specifications for Food Grade PGX®

Specifications provided in Table 4 for bulk PGX® include viscosity, lead, arsenic, sodium, potassium, bacteria, yeast and mold, and the absence of *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*.

Table 4. Specifications for PGX®

Analysis	Method	Specification	Batch Analysis Results (n=5) Range	Average
Viscosity	5 g in 350 ml H_2O; Reading in one hour*	>35,000 cP		
Loss on drying (%)	135°C oven	NMT 15	4.20 – 9.08	6.66
Lead (ppm)	ICP	NMT 2	<0.50 – 0.92	0.62
Arsenic (ppm)	ICP	NMT 3	<1.5	<1.5
Sodium (%)	ICP		2.34 – 2.94	2.58
Potassium (%)	ICP	NMT 4	0.41 – 0.80	0.60
Microbiological (cfu/g)				
Standard plate count	USP<61>	NMT 1000	20 - 700	256
Yeast & Mold (cfu/g)	USP<61>	NMT 100	<10	Negative
Escherichia coli	USP<61>	Negative	Negative	Negative
Salmonella	USP<61>	Negative	Negative	Negative
Staphylococcus aureus	USP<61>	Negative	Negative	Negative

*Viscosity is measured on a Brookfield type rotational viscometer at room temperature; cfu = colony forming units; cP = centipoise; g = grams; ICP = inductively coupled plasma; n = number of batches analyzed; NMT = not more than; ppm = parts *per* million; USP = United States Pharmacopeia; methods available upon request

3. Self Limiting Levels of Use

The quantity of PGX® is self-limiting to the extent that it creates a sensation of fullness, similar to any of the conventional soluble fiber food ingredients.

4. Basis of GRAS Determination

The determination that PGX® is GRAS is on the basis of scientific procedures, as outlined in the attached Dossier in Support of the Generally Recognized as Safe (GRAS) Status of PGX® as a Food Ingredient. On the basis of the data and information described in the attached dossier, the amendment to the GRAS, and other publicly available information, there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that PGX® is GRAS under the intended conditions of use.

Pages 000016-000019 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

January 8, 2009

FINAL

Panel Members

Julie Miller-Jones, Ph.D.

I. Glenn Sipes, Ph.D.

John Thomas, Ph.D.

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

FINAL

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

1. EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by InovoBiologic Inc. (hereafter referred to as Inovo) to determine the Generally Recognized As Safe (GRAS) status, of PolyGlycopleX® (hereinafter referred to as PGX) based on scientific procedures. PGX is to be used as an ingredient to add fiber to the diet, such that total daily consumption of PGX from foods and dietary supplements described herein will be 13,070 mg *per* day. In particular, the Expert Panel has evaluated the proposed use of PGX at specified levels in the foods listed in Table 5 of this document. A comprehensive search, conducted by Burdock Group, of the scientific literature for safety information on PGX and related compounds was conducted through September 2008 and, along with supporting documentation, was made available to the Expert Panel. InovoBiologic, Inc. assures that all relevant, unpublished information in its possession has been supplied to Burdock Group and has been summarized in this monograph. The toxicology and safety-in-use associated with PGX were critically evaluated. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

2. INTRODUCTION

Dietary fiber is defined as nondigestible carbohydrates and lignin that are intrinsic and intact in plants (IOM, 2005). A more detailed definition has been coined by the American Association of Cereal Chemists (AACC, 2001):

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[1] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.

> Dietary fiber is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber exhibits one or more of either laxation (fecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation.

"Functional fiber" is defined as isolated nondigestible carbohydrates that contain physiological properties such as fermentation, water-holding capacity, viscosity and bile acid binding, potentially providing beneficial effects to humans (Schneeman, 1985; Blackwood *et al.*, 2000; IOM, 2005). Fiber includes isolated or extracted nondigestible carbohydrates, produced by either or a combination of chemical synthesis, enzymatic, or aqueous extraction means, which are reported to have beneficial physiological effects in humans (Ludwig *et al.*, 1999; Liu *et al.*, 2003; IOM, 2005). Synthetically manufactured oligosaccharides (of three or more degrees of polymerization), manufactured resistant starch, and naturally occurring polysaccharides or oligosaccharides extracted from their source and modified (*e.g.*, altered to a shorter polymer length or a different molecular arrangement) are included in the definition of fiber. Nondigestible monosaccharides, disaccharides, and sugar alcohols are instead considered "sugars" or "sugar alcohols", as they fall under the term "carbohydrates" on a food label (IOM, 2005). Notwithstanding the foregoing, fiber, regardless of the motivation for consumption, is still fiber, whose differences are based on physiologic outcomes as a product of carbohydrate composition and type of bonding, rather than the motivation for consuming it. The type of carbohydrate composition and bonding that results in formation of viscous fiber, when wetted and ingested, may delay gastric emptying time, resulting in a prolonged sensation of "fullness". Delayed gastric emptying may also reduce postprandial blood glucose concentrations, potentially increasing glucose sensitivity. Viscous fiber may also interfere with the absorption of dietary fat and cholesterol, and decrease enterohepatic recirculation of cholesterol and bile acids, with an overall result of decreased concentration of blood cholesterol. Dietary carbohydrates that are slowly digested or pass through the system relatively undigested are reported to decrease the amount of insulin required and may benefit those with metabolic syndrome, obesity, or diabetes (Wong *et al.*, 2006). Dietary fiber may influence several parameters affecting metabolic

syndrome, including the modification of gut peptides involved in appetite and glucose homeostasis, influence food intake, blood glucose, insulin, and lipid levels (French, 2004; Delzenne and Cani, 2005). Another well known effect of fiber consumption, especially those fibers that are poorly digested, is the improvement of fecal bulk and laxation and a decrease in the occurrence of constipation, although additional research is necessary to determine the extent of the benefits (Tan and Seow-Choen, 2007). It has been reported that compared to the 1950s, present-day consumers are eating substantially less starch and fiber (Prynne *et al.*, 1999). The Institute of Medicine (IOM) (2005) has recommended that women consume up to 25 g fiber *per* day, and men consume up to 38 g fiber *per* day, as part of a healthy diet. However, estimates indicate that dietary fiber intakes range from 12.1 – 13.8 g/day for women and 16.5 – 17.9 g/day for men (IOM, 2005).

PGX is manufactured from three water soluble polysaccharides: konjac powder,[2] sodium alginate, and xanthan gum. The polysaccharides used in its production are complementary to each other and act synergistically to form strong bonds that lead to a level of viscosity that is 3 – 5 times higher than any other known polysaccharide (InovoBiologic, 2007a). Viscous fibers thicken when mixed with fluids and have been associated with altered cholesterol and blood glucose concentrations, slower transit time through the small intestine, and prolonged gastric emptying (Dikeman and Fahey, 2006). Konjac glucomannan (*i.e.*, konjac) is a powder obtained form the tuber of the *Amorphophallus* konjac plant c. Koch, a member of the Araceae family that originated in east Asia (Shelso, 1989; Nishinari *et al.*, 1992). Konjac is composed mainly of glucomannan, a soluble, fermentable, highly viscous fiber (Keithley and Swanson, 2005). Glucomannan is a polysaccharide chain of *beta-D*-glucose and *beta-D*-mannose with attached acetyl groups in a molar ratio of 1:1.6 with *beta*-1,4 linkages (Shimahara *et al.*, 1975). Human salivary and pancreatic enzymes cannot cleave *beta*-1,4 linkages, so glucomannan passes relatively unchanged to the colon, where resident bacteria are able to ferment the glucomannan to the short chain fatty acids (SCFA) acetate, propionate, and butyrate. SCFA production has been associated with decreased pH, leading to a potential in the reduction of pathogenic

[2] Konjac powder is also known as konjac flour.

clostridia, reduced ammonia absorption, decreased bile acid solubility, and increased mineral absorption (Wong *et al.*, 2006). SCFA may also modulate glucagon-like peptide-1 (GLP-1), which stimulates insulin release and increases insulin-independent glucose uptake. Dietary fiber intake significantly alters proglucagon gene expression and modulates GLP-1 and insulin secretion (Reimer and McBurney, 1996; Massimino *et al.*, 1998; Keenan *et al.*, 2006). SCFA also bind receptors in adipose tissues and may stimulate leptin production; leptin is a potent anorexigenic hormone that may indirectly suppress food intake (Hirasawa *et al.*, 2008). Prolonged carbohydrate absorption from a primary meal may result in a reduced tendency for subsequent low blood sugar levels, and a smaller glucose regulatory response and improved glucose uptake after the second meal, which has been termed the "second-meal" effect (Wolever *et al.*, 1988). Sodium alginate is a polyuronic saccharide-based soluble fiber isolated from the cell walls of various brown seaweed species around the world, and is also produced as an extracellular matrix by certain bacteria (Brownlee *et al.*, 2005). It is a linear glycuronoglycan polymer that contains 1:4-linked β-*D*-mannuronic and α-*L*-guluronic acid residues (Burdock, 1997). Xanthan gum is a bacterial polysaccharide-based soluble fiber from *Xanthomonas campestris* NRRL B-1459, and is composed of *D*-glucose, *D*-mannose, *D*-glucuronic acid, acetic acid, and pyruvic acid (Sloneker and Jeanes, 1962; Torrestiana *et al.*, 1990).

PGX can be used as an ingredient to provide consumers with a source of fiber in their diets. This dossier is a summary of the scientific evidence that supports the general recognition that PGX as safe for human consumption as a food ingredient.

2.1. History of Use

PGX *per se,* does not have a history of use as a food ingredient in the United States, although konjac, sodium alginate[3] and xanthan gum[4] have been utilized in the US as food

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[3] Title 21 of the Code of Federal Regulations, Part 184- Direct Food Substances Affirmed as Generally Recognized as Safe. Subpart B -Listing of Specific Substances Affirmed as GRAS, Section 184.1724, Sodium alginate.

ingredients for several decades. Konjac has obtained GRAS status as a meat binder, and has been utilized in Asian countries for hundreds of years as flour (JECFA, 2007a).

2.2. Current Uses

Sodium alginate and xanthan gum are currently used in foods as emulsifiers, firming agents, flavor enhancers, flavor adjuvants, processing aids, stabilizers, and thickeners. Konjac powder may be utilized as a binder in meat and poultry products. PGX is an additional source of fiber in the diet.

2.3. Regulatory Status

PGX is not regulated by the FDA. The regulatory status of the primary ingredients (*i.e.*, konjac powder, sodium alginate, and xanthan gum) is summarized in Table 1.

Konjac powder (also known as konjac flour) has been reviewed by JECFA (1996), and an ADI of "Not Specified" was established, with the permitted functionality as a thickener, emulsifier, stabilizer, gelling agent, texturizer, and glazing agent.[5] A review of publicly available FDA databases failed to locate any FDA regulations pertaining to the use of konjac flour. The USDA, through the Food Safety and Inspection Service (FSIS), published in the Federal Register a final rule, wherein konjac flour was permitted for use as a binder in meat and poultry products in which starchy vegetable flours are permitted, at levels not to exceed 3.5% of the product formulation, individually or collectively with other binders. FSIS indicated that the use of konjac flour for this purpose was presented in an acceptability determination letter (USDA, 2004). Publicly available documentation indicates that a GRAS determination for konjac flour has been submitted to FDA for affirmation as "GRAS"; although there is no further information on this submission (CFSAN, 1995). A mini-cup gel candy sold as individual, mouth-sized servings that contained konjac was issued a warning by FDA, due to a potential risk for choking in infants,

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[4] Title 21 of the Code of Federal Regulations, Part 172- Food Additives Permitted for Direct Addition to Food for Human Consumption, Subpart G-Gums, Chewing Gum Bases and Related Substances, Section 172.695, Xanthan gum.

[5] Konjac flour; http://www.inchem.org/documents/jecfa/jeceval/jec_1250.htm; site visited April 6, 2007.

children and the elderly.[6] However, this choking risk was specific to the unique formulation and final size of the product, and this choking hazard is not considered relevant to the formulation of PGX added to food products as stated in this GRAS.

Sodium alginate is regulated by the FDA for use as a texturizer, stabilizer, thickener, and formulation aid at a maximum use level of 1.0% in condiments and relishes and 6.0% in pimento ribbon for stuffed olives; a stabilizer and thickener at a maximum level of 0.3% in confections and frostings; 4.0% as a firming agent, flavor adjuvant, stabilizer, and thickener in gelatins and puddings; 10.0% in hard candy as a stabilizer and thickener; 2.0% as a formulation aid and texturizer in processed fruits and fruit juices; and at a maximum level of 1.0% as an emulsifier, firming agent, flavor enhancer, flavor adjuvant, processing aid, stabilizer, and thickener, in all other foods. JECFA allows for use of sodium alginate as a stabilizer, thickener, gelling agent, and emulsifier, with an Acceptable Daily Intake (ADI) of "Not Specified."[7]

Xanthan gum is regulated by the FDA for use as a stabilizer, thickener, emulsifier, suspending agent, bodying agent, or foam enhancer, used or intended for use in accordance with good manufacturing practice in foods for which standards of identity established under section 401 of the Act do not preclude such use.[8] JECFA has stated an ADI for xanthan gum as "Not Specified", for use as a thickener, stabilizer, emulsifier, and foaming agent.

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[6] http://www.fda/gov/bbs/topics/NEWS/2001/NDS00770.html; site visited December 11, 2008.

[7] JECFA has indicated an ADI of "not specified" as a term applicable to a food substance of very low toxicity which, on the basis of the available data, the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bound of good manufacturing practice, *i.e.*, it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance; http://www.who.int/ipcs/food/jecfa/glossary.pdf; site visited April 6, 2007.

[8] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2006.

Table 1. Regulatory status of the PGX ingredients konjac powder, sodium alginate, and xanthan gum

Ingredient	Agency	Permitted functionality	Use limits (maximum level of use in food)	Reference
Konjac powder	USDA	Binder in meat and poultry products in which starchy vegetable flours are permitted	Not to exceed 3.5% of the product formulation individually or collectively with other binders	9 CFR Chapter III[9]
Konjac powder	JECFA	Thickener, emulsifier, stabilizer, gelling agent, texturizer, and glazing agent	An ADI of "Not Specified" was established	JECFA (2007a)
Sodium alginate	FDA	Texturizer, formulation aid, stabilizer, thickener, firming agent, flavor adjuvant, emulsifier, flavor enhancer, surface active agent	Condiments and relishes, 1.0%; Pimento ribbon for stuffed olives, 6.0%; confections and frostings, 0.3%; gelatins and puddings, 4.0%; hard candy, 10.0%; processed fruits and fruit juices, 2.0%; all other food categories, 1.0%	21 CFR § 184.1724[10]
Sodium alginate	JECFA	Stabilizer, thickener, gelling agent, and emulsifier	An ADI of "Not Specified" was established	JECFA (2007b)
Xanthan gum	FDA	Stabilizer, emulsifier, thickener, suspending agent, bodying agent, or foam enhancer	The additive is used or intended for use in accordance with good manufacturing practice in foods for which standards of identity established under section 401 of the Act do not preclude such use.	21 CFR § 172.695[11]
Xanthan gum	JECFA	Thickener, stabilizer, emulsifier, foaming agent	An ADI of "Not Specified" was established	JECFA (2007c)

ADI = Acceptable Daily Intake; CFR = Code of Federal Regulations; FDA = United States Food and Drug Administration; JECFA = Joint FAO/WHO Expert Committee on Food Additives; USDA = United States Department of Agriculture

[9] United States Department of Agriculture. Food Safety and Inspection Service (FSIS) Directive 7120.1 (2002); http://www.fsis.usda.gov/oppde/rdad/fsisdirectives/7120.1.htm; site visited June 15, 2006.
[10] Title 21 of the US Code of Federal Regulations (CFR), section 184.1724, 2006
[11] Title 21 of the US Code of Federal Regulations (CFR), section 172.695, 2006

2.4. Proposed Use or Uses

PGX will be added to food to provide a source of fiber in the diet.

3. DESCRIPTION, SPECIFICATIONS AND MANUFACTURING PROCESS

3.1. Description and Specifications

PGX is an off-white granular powder composed of a proprietary agglomeration of the food-grade water-soluble polysaccharides konjac powder, sodium alginate, and xanthan gum. These polysaccharides are processed in a conditioned chamber to produce an interlocking matrix of the three components (*i.e.*, the agglomeration). The polysaccharides used in the production of PGX act synergistically to form strong bonds that lead to a level of viscosity that is 3 – 5 times higher than the individual components or any other known individual polysaccharide. This agglomeration results in a new and separate ingredient, rather than only a blend (or mixture) of the three separate components. Two polysaccharides are complementary to each other and have the ability to hook together, while the third polysaccharide in the PGX blend fastens the other two polysaccharides together. In principle, when the polysaccharide complex of a soluble polysaccharide agglomeration interacts with liquid and nutrients, it swells due to the highly hydroscopic property. A critical point is reached once swollen, and the polysaccharide matrix breaks apart, releasing the three individual polysaccharide coils which induces a highly viscous, stable gel matrix that incorporates the liquid and nutrients in the solution[12]. The viscosity of a soluble polysaccharide is directly related to its physiological effects (Jenkins *et al.*, 1978). In general, when fiber is mixed with food and human digesta in the gut, a firm soluble polysaccharide/food matrix is formed, causing nutrients to be slowly absorbed from the small intestine (Jenkins *et al.*, 1995; IOM, 2005). The physical and chemical properties and specifications for PGX are provided in Table 2 and Table 3, respectively.

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[12] InovoBiologic, Personal communication.

Table 2. Physical and chemical properties of PGX

Characteristic	*Percentage* of individual ingredient in PGX
Konjac powder	48-90
Sodium alginate	5-30
Xanthan gum	5-20
Appearance	Granular
Color	Off-white
Particle size	NMT 1% 20 mesh; 60% 40 mesh; NMT 35% 60 mesh; NMT 4% fines less than 60 mesh

Table 3. Specifications of PGX (InovoBiologic, 2007b)

			Batch Analysis Results (n=5)**	
Analysis	Method	Specification	Range	Average
Carbohydrates (%)	Calculation	NLT 75	80 - 85.4	83.6
Total dietary fiber (%)	AOAC 991.43	NLT 75	82.4 - 91.6	84.74
Ash (total) (%)	AOAC 920.155c	NMT 7	5 – 6.2	5.72
Protein (%)	AOAC 981.10 (modified)	NMT 7	1.8 – 2.2	2.04
Fat (%)	AOAC 925.32	NMT 1	<0.1 – 0.1	<0.1
Viscosity	5 g in 350 ml H_2O; Reading in one hour*	>35,000 cP		
Loss on drying (%)	135°C oven	NMT 15	4.20 – 9.08	6.66
Lead (ppm)	ICP	NMT 2	<0.50 – 0.92	0.62
Arsenic (ppm)	ICP	NMT 3	<1.5	<1.5
Sodium (%)	ICP		2.34 – 2.94	2.58
Potassium (%)	ICP	NMT 4	0.41 – 0.80	0.60
Microbiological (cfu/g)				
Standard plate count	USP<61>	NMT 1000	20 - 700	256
Yeast & Mold (cfu/g)	USP<61>	NMT 100	<10	Negative
Escherichia coli	USP<61>	Negative	Negative	Negative
Salmonella	USP<61>	Negative	Negative	Negative
Staphylococcus aureus	USP<61>	Negative	Negative	Negative

*Viscosity is measured on a Brookfield type rotational viscometer at room temperature; **Individual batch analyses are provided in APPENDIX B; AOAC = Association of American Chemists; cfu = colony forming units; cP = centipoise; g = grams; ICP = inductively coupled plasma; n = number of batches analyzed; NLT = not less than; NMT = not more than; ppm = parts *per* million; USP = United States Pharmacopeia; methods available upon request

3.2. Manufacturing Process

PGX is manufactured by a blending, followed by agglomeration, of food grade konjac, sodium alginate, and xanthan gum. A graphical depiction of the manufacturing process is offered in Figure 1. To summarize, the raw materials are first tested against internal specifications, and those that meet specifications and are safe and suitable for this application, are weighed and blended using a mechanized blender. The blended material is then agglomerated with water, and subsequently dried and tested against finished product specifications. Product that meets the required specifications is released for sale. Product that does not meet the specifications is discarded. The agglomerated powder that meets the required particle size specifications is packaged.

The final product, PGX, is available as an off-white powder packed and stored at 25°C.

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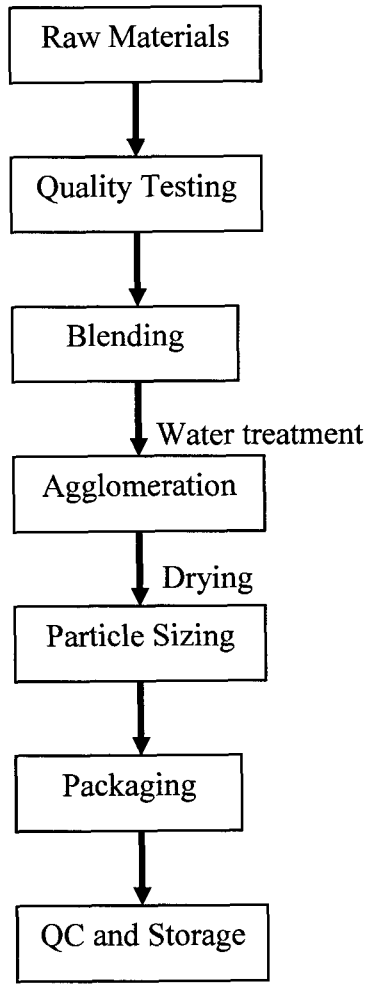

Figure 1. PGX production scheme

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3.3. Stability

PGX is stable for two years from the date of manufacture when stored in tight containers under a cool, dry environment, away from direct sunlight (Table 4) (InovoBiologic, 2005).

Table 4. Stability of PGX over time (InovoBiologic, 2008)

	Viscosity (cps) specification*	Viscosity (cps) measured	Comments
Lot 1088050727			
Time 0	>35,000	>90,000	
Time 37 months	>35,000	>90,000	Conforms
Lot 1285060330			
Time 0	>35,000	61,984	
Time 12 months	>35,000	60,700	
Time 28 months	>35,000	65,570	Conforms
Lot 1284060330			
Time 0	>35,000	74,450	
Time 28 months	>35,000	78,950	Conforms

* Viscosity is measured on a Brookfield type rotational viscometer at room temperature; conforms = measurement meets or exceeds the stated specification for the parameter; cps = centipoise

4. ESTIMATED DAILY INTAKE

An understanding of the consumption of the new proposed uses of the food ingredient, as an index of consumer exposure at ingredient use level(s) in various food group(s), is key to any food safety risk assessment process. Pursuant to this end, Inovo provided the details regarding the intended uses and use levels. The food groups as defined by the FDA (21 CFR 170.3(n)) are listed in Table 5. Individual food products within these categories were selected (APPENDIX A), and a consumption analysis was performed. Inovo indicated that PGX would be added to specific foods (APPENDIX A) at 2.5g/serving.

The intake profile (amount and frequency) by individuals in the NHANES dietary database[13] was used to calculate the estimated daily intake (EDI) of PGX for individuals consuming the food groups selected for the addition of PGX *per* this GRAS evaluation.

[13] Source: HHS What We Eat in America, National Health and Nutrition Examination Survey (NHANES) 2003-2004, USDA

Estimated daily intake of PGX from its proposed use in food can be calculated by multiplying the concentration of PGX in food (mg *per* g food) and the amount of food consumed (g *per* day). The consumption of food varies according to the consumption patterns of the individual. The estimated daily intake is typically calculated for the "average" consumer and the "high" consumer. For the purpose of safety assessment, a "high" consumer is considered so that exposure is not underestimated. For purposes of the estimate, this subgroup is generally represented by the consumers of a given food at the 90th percentile in an "eaters-only" population. Based on the addition of PGX to the foods specified and the concentrations provided in Table 5, a mean intake of 5019 mg/day PGX was calculated, with the 90th percentile consumption at 10,070 mg/day (167.8 mg/kg/day for a 60 kg person).

Table 5. Food groups selected for PGX supplementation*

Food Category	Intended use level (ppm)
Yogurts	11,110
Milk shakes and fruit smoothie-type drinks	10,420
Frozen yogurt, ice cream bar, and puddings	6,670
White and whole wheat breads	50,000
Cookies	83,330
Breakfast bars	62,500
Granola-type bars	62,500
Noodles	17,860
Whole wheat cereals	83,330
Lasagna and macaroni/cheese	10,000
Fruit juices and fruit juice bars	10,420
Cereal beverage	10,420

*The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing in 21 CFR 170.3(n) for that food category.
ppm=parts *per* million

Another possible source of PGX in the diet is from consumption of dietary supplements. PGX consumption from dietary supplements is difficult to determine. The 1987 National Health Interview Survey identified that 51.1% of the adults aged 19-99 years in the US consumed a vitamin/mineral supplement in the past year, but that only 23.1% did so daily (Subar and Block, 1990). Multivitamins were the most commonly consumed supplement at that time. PGX is

available as a dietary supplement,[14] with one serving size providing 1000 mg PGX, with the suggested serving consumed prior to meals. Since there are no available statistical data on consumption of dietary supplements that are a source of PGX, we have used the levels reported in labeling as a basis for estimating consumption ranges. Thus, the potential theoretical PGX consumption from dietary supplements may equal 3000 mg/day. The total PGX consumption as an added food ingredient and as a dietary supplement at the 90th percentile is estimated to be 13,070 mg/day, or 217 mg/kg/day for a 60 kg person (Table 6).

Table 6. PGX: Predicted intake following supplementation of selected foods at the indicated levels (Table 5) and dietary supplements, and total intake (predicted + dietary supplement) for individuals consuming selected supplemented foods and dietary supplements

	Per User (mg/day)	
PGX intake from:	Mean	90th Percentile
Possible maximum consumption with PGX as an added ingredient to food	5019	10,070
Possible consumption of PGX as a dietary supplement*	3000	3000
Total from all sources (food + dietary supplements)	8019	13,070

*Consumption according to label directions

5. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME)

In general, insoluble fiber is indigestible and passes through the body virtually intact; it possesses passive water-attracting properties to increase bulk and shorten transit time through the intestine.[15] Soluble fiber, although resistant to digestion and absorption in the human small intestine, undergoes complete or partial fermentation in the large intestine (AACC, 2001). In preclinical studies, the amount of fermentation has been variable, depending on the model utilized, as indicated below.

PGX is an agglomeration of the three soluble fibers: konjac powder, sodium alginate, and xanthan gum. Sharratt and Dearn (1972) found that the radiolabeled alginic acid moiety of

[14] http://www.smartbomb.com/nutfac3554.html; site visited September 18, 2008
[15] http://www/cfsan.fda.gov/~dms/fdafiber.html; site visited September 18, 2008

propylene glycol alginate was not absorbed from the gastrointestinal tract of albino CF1 mice. The autoradiographs indicated that the radioactivity was confined to the gastrointestinal tract at all times up to five days after dosing. This study confirmed studies completed in rats administered a diet containing 10% ^{14}C-labeled sodium alginate, which found only negligible levels of absorption (Humphreys and Triffitt, 1968). Konjac is degraded almost 100% by soluble human fecal enzymes to release 4-*O*-β-*D*-mannopyranosyl-*D*-mannopyranose (β-1,4-*D*-mannobiose), 4-*O*-β-*D*-glucopyranosyl-*D*-gluco-pyranose (cellobiose), 4-*O*-β-*D*-glucopyranosyl -*D*-manno-pyranose, and minor amounts of mannose and glucose (Nakajima and Matsuura, 1997; Matsuura, 1998). The authors proposed that the enzymes originated from intestinal bacteria, with these three disaccharides further degraded by cell-associated enzymes to glucose or mannose, finally absorbed or fermented by intestinal anaerobic bacteria to produce formic acid, acetic acid, propionic acid, and 1-butyric acid. These fatty acids are absorbed across the intestinal wall and used as a source of energy (Pomare, 1985). Xanthan gum has been reported not to be utilized by the body, as a study in rats found that practically all of the gum fed during a seven-day period could be accounted for in the feces (Booth *et al.*, 1963). A summary of an unpublished report noted that ^{14}C-radiolabeled xanthan gum fed to rats at 2% of the diet (approximately 2000 mg/kg) resulted in the formation of carbon dioxide, with approximately 98% of the radioactivity in the feces attributed to unchanged or slightly modified polysaccharide, and no accumulation in the tissues (JECFA, 1987).

Ingestion of fiber has been shown to alter intestinal microflora. Fujiwara *et al.* (1991) evaluated the effects of dietary konjac ingestion on intestinal microbial metabolism and microflora composition in conventional male F344 rats and C3H/He male mice bearing human flora. Konjac at 10% (w/w)[16] of the diet was fed to the rats (n = 13 *per* group) and mice (n = 5 *per* group) for two months (approximately equivalent to 10,000 mg/kg/day in rats and 15,000 mg/kg/day in mice, respectively), during which time feces were collected and enzymes (fecal β-glucuronidase, nitroreductase, and azoreductase activities), soluble protein, and putrefactive products (mainly, the tyrosine and tryptophan metabolites *p*-cresol, indole, and skatole) were

[16] Weight/weight ratio

evaluated. Konjac did not significantly alter body-weight gain, fecal water, or fecal protein content in F344 rats, but fecal indole and fecal β-glucouronidase, nitroreductase and azoreductase activities were significantly suppressed. In the C3H/He mice bearing human flora, the composition of the flora of the mice that were fed the control diet was similar to that of the original human feces used as the source of the inoculum. Konjac consumption significantly decreased the number of streptococci ($P<0.05$), but at the genus level, there were no differences in total counts or in counts for other bacterial or fungal groups, between konjac-fed and control-fed mice. Fecal β-glucuronidase and nitroreductase activities in the konjac-fed group were significantly decreased, compared to the control group ($P<0.01$ and $P<0.05$, respectively), while azoreductase activity was unchanged. Konjac ingestion significantly lowered the fecal putrefactive products *p*-cresol and indole ($P<0.01$), and reduced skatole to below the limit of detection. Fujiwara *et al.* (1991) concluded that in C3H/He male mice bearing human flora, dietary konjac may modify microbial metabolism without causing significant alterations in the intestinal microflora composition.

The ingestion of soluble fiber diets increases fecal volume and speeds gastrointestinal transit time, but delays gastric emptying, which may induce satiety and provide a protective role on the gastrointestinal tract. The effects of konjac consumption on the cecum and colon were investigated in rats consuming diets containing 20% konjac (approximately equal to 20,000 mg/kg/day) for eight weeks (Konishi *et al.*, 1984). Male Wistar rats ($n = 6$ *per* group) were fed diets containing 20% konjac or corn starch (control), after which the rats were weighed, and the cecum and colon were retained, rinsed with saline, blotted, weighed, and homogenated for DNA, RNA, protein, and sodium/potassium ATPase activity. Konjac consumption by rats for eight weeks resulted in a significant decrease in body weight gain ($P<0.05$), but increased the cecum wet weight[17] and cecum weight as a *percent* of body weight ($P<0.001$), as well as the colon wet weight ($P<0.01$) and colon weight as a *percent* of body weight ($P<0.05$). The level of sodium/potassium ATPase activity was proportional to the tissue enlargement, and significantly greater than control values ($P<0.001$). The authors concluded that the enlargement of the cecum

[17] Wet weight = wet of the whole tissue

and colon induced by konjac ingestion resulted from hypertrophic hyperplasia, an increase in both number and size of the mucosal cells. A positive correlation between fecal excretion and colonic weight was found in female Wistar rats fed indigestible polysaccharides, in which the authors assumed that the increased colonic work needed to propel the more bulky contents along the large intestine may lead to muscle hypertrophy (Elsenhans *et al.*, 1981).

As high fiber diets may alter the absorption of inorganic ions, Fei (1993) evaluated the effect of konjac consumption on zinc, iron, and calcium absorption rates in rats. Sprague-Dawley rats (5 male and 5 female rats *per* group) were administered (*via* the diet) 0.5%, 1%, 5%, or 10% konjac for six weeks (approximately equivalent to 500, 1000, 5000, and 10,000 mg/kg/day, respectively). Zinc, iron, and calcium content of the urine, feces, and feed were quantified every two weeks. Serum hemoglobin and erythrocyte free protoporphyrin contents were analyzed after the six-week study. Serum hemoglobin was significantly lower in the 10% konjac group, compared to controls ($P<0.05$). Protoporphyrin content was not altered. The absorption rate of zinc was decreased in the 5% and 10% konjac groups at the end of the second week ($P<0.05$), but returned to normal values by the fourth week. Iron and calcium levels were not altered with konjac consumption at any dose level.

Oku *et al.* (1982) found that administration of konjac (for seven or eight weeks) to male Wistar rats at 10 or 20% of the diet (approximately equivalent to 10,000 or 20,000 mg/kg bw[18]/day, respectively) significantly decreased body weight gain ($P<0.05$ and $P<0.01$, respectively), and bone ash levels ($P<0.05$ and $P<0.01$, respectively). Food intake by the groups consuming konjac was not different from controls. Calcium transport by the everted gut sac and calcium binding protein from homogenated duodenum tissue were decreased ($P<0.01$) in the 20,000 mg/kg/day konjac dose group, compared to controls.

In a clinical study, human subjects (n = 12) gradually consumed increasing levels of konjac over a six day period (3000 mg/day increased to 9000 mg/day), with the highest dose consumed for a three-day period. Zinc, iron, and calcium content and the apparent digestibility of zinc, iron and calcium (fecal and serum levels) in each individual were calculated on the third

[18] Body weight

and ninth day of consumption. No effects were seen on the absorption of zinc, iron, or calcium in these healthy adults over a six-day period (Fei, 1993).

Bosscher *et al.* (2001) evaluated the effects of the addition of thickening agents, including alginic acid (the acid of sodium alginate salt), on the availability of calcium, iron, or zinc from whey-based infant formulas, in a dialysis *in vitro* method adapted to simulate the conditions of infants younger than six months).[19] Supplementation of 2 g of alginic acid-based agents *per* 100 ml whey formula depressed calcium availability from 13.3% to 5.3% ($P<0.05$) (the overall consumption of alginic acid was not stated). Iron and zinc availabilities increased from 1.28% to 6.05%, and from 6.7% to 10.2%, respectively ($P<0.05$); although Geisser (1990) reported that sodium alginate reacts with iron(II) or iron(III)-salts *in vitro* at pH values of 3.0, 5.5 and 8.0, rendering the bound iron biologically unavailable. Kikunaga *et al.* (1999) reported that Wistar rats fed a diet containing 31 or 63 g/kg sodium alginate *ad libitum* (approximately equivalent to 3100 and 6300 mg/kg bw/day, respectively) for 28 days decreased food intake and body weight gain. These changes were accompanied by significant decreases in kidney magnesium and calcium levels, but not serum levels of these minerals. Magnesium and calcium absorption and retention values[20] were significantly decreased in rats administered sodium alginate, compared to the control group. In contrast, sodium alginate did not interfere with the absorption of calcium in healthy adults ($n = 6$) fed 8 g sodium alginate *per* day for seven days (Millis and Reed, 1947). These data indicate that there is conflicting evidence on whether soluble fiber products at very high consumption levels may alter the absorption of various minerals in preclinical studies, although consumption in a clinical trial at a lower level did not affect zinc, calcium, or iron absorption.

A review by Furda (1990) indicated that high fiber diets may also affect cholesterol and lipid uptake. Kodama *et al.* (1972) found that konjac, when fed to male albino Wistar rats at 5%

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[19] This *in vitro* method contains a gastric phase and an intestinal phase to simulate intestinal absorption by infants. In the gastric phase, a solution of pepsin is added to the sample, incubated and the titratable acidity was measured. In the intestinal stage, a small dialysis bag containing $NaHCO_3$ was added to the cell, which results in a gradual pH change from acid to neutral. After 30 min, a pancreatin/bile mixture was added and the sample was dialyzed.

[20] Absorption means apparent absorption [(Intake –Fecal loss)/Intake x100]; Retention = (Intake – Fecal loss – Urinary loss).

of the diet for six days (approximately 5000 mg/kg bw/day) resulted in significant decreases in plasma and liver cholesterol levels ($P<0.05$), when compared to controls. Kodama *et al.* (1972) further reported that konjac significantly decreased the amount of dietary cholesterol retained in the viscera, small intestinal wall, blood plasma and liver tissue ($P<0.05$) in male albino Wistar rats fed 5000 mg/kg bw/day konjac for six days. The authors concluded that konjac may interfere with the absorption of dietary cholesterol through the inhibition of cholesterol solubilization.

Ebihara *et al.* (1981a; 1981b) evaluated the effects of konjac on glucose absorption in Wistar rats. An acute gavage dose of konjac (0.5%, approximately equivalent to 500 mg/kg bw) concurrent with glucose (20% solution) administration significantly decreased the early rise in plasma glucose and insulin concentrations, as determined at 10, 20, and 30 minutes after administration. Konjac administration (*via* gavage in a 1.0% solution, approximately equivalent to 1000 mg/kg) concurrent with glucose (20% w/v)[21] significantly delayed gastric emptying, compared to the control diet. When a 10 cm jejunal segment was perfused *in vitro* with a 2% glucose solution with or without added konjac (at 1%) at a rate of 5 ml/0.5 hour, glucose absorption was significantly decreased in the presence of konjac in the perfusate, when compared with the control segment. Konjac was also found to significantly interfere with glucose diffusion. Ebihara *et al.* (1981a; 1981b) concluded that the decreased levels of plasma glucose related to the combined effect of konjac on delaying the gastric emptying time and decreased intraluminal glucose diffusion, which resulted in delayed glucose absorption.

Consumption of sodium alginate *via* the diet (10% of the diet, approximately equivalent to 10,000 mg/kg bw/day) was evaluated in CD albino rats ($n=6$) for alginate-induced changes in the absorption and utilization of protein, fat, and calcium in young rats (Viola *et al.*, 1970). Sodium alginate ingestion reduced food intake and weight gain ($P<0.01$), but only slightly impaired the absorption of protein and lipids. Calcium absorption was depressed by approximately 25%, compared to controls, although this was not a significant reduction. One-third of the sodium alginate was isolated in the feces, and the digestible energy values were positive, indicating partial digestion of the sodium alginate. Sodium alginate (2.5%,

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[21] Weight/volume ratio

approximately 2500 mg/kg/day for up to 28 days) has also been found to reduce food intake through the gel formation of the alginate with dietary calcium in the stomach in streptozotocin-induced diabetic Sprague-Dawley rats, significantly reducing the postprandial glycemic response (Ohta *et al.*, 1997).

In summary, published scientific findings on the components of PGX indicate that these soluble polysaccharides are not digested in the small intestine, but are partially digested by resident microbial flora in the lower gastrointestinal tract. The partial digestion may result in the formation of carbon dioxide and/or short chain fatty acids, which are absorbed and utilized as a source of energy, albeit likely minimal in caloric value. The scientific literature indicates that while there is a modest alteration in bacterial flora with these three different fiber types, there are no reports of significant changes with any of the three. Conflicting evidence has been published on the ability of large intakes of fiber to alter mineral absorption. A summary of the data stated here is presented in Table 7.

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Table 7. Summary of effects of konjac powder, sodium alginate or xanthan gum on ADME

Material	Dose/Concentration	Study Objective	Species	Results	Reference
Konjac powder	Konjac powder (0.25%) (w/v) in acetate buffer	*In vitro* Absorption and degradation assays	Human fecal enzymes	Konjac is degraded almost 100% by fecal enzymes, ultimately degraded to produce formic, acetic, propionic, and 1-butyric acids which are absorbed as energy sources	Matsuura (1998); Nakajima and Matsuura (1997)
Konjac powder	Konjac in the diet: 10,000 mg/kg/day in rats for two months; 15,000 mg/kg/day in mice for three months	Effect of konjac on fecal parameters or energy absorption	F344 rats; C3H/He mice with human fecal flora	Feeding konjac did not alter body-weight gain, fecal water, or fecal protein content in F344 rats. Konjac did not change mouse flora, but significantly reduced β-glucuronidase, nitroreductase activities, and p-cresol, indole, and skatole	Fujiwara *et al.* (1991)
Konjac powder	20,000 mg/kg/day in the diet for eight weeks	Alterations of cecum and colon physical parameters	Wistar rats	Konjac consumption for eight weeks significantly decreased body weight gain but increased cecum and colon wet weight and *percent* of body weight, resulting from hypertrophic hyperplasia	Konishi *et al.* (1984)
Konjac powder	0, 500, 1000, 5000 and 10,000 mg/kg/day for six weeks	Effect of high fiber diets on inorganic ion absorption	Sprague-Dawley rats	Serum hemoglobin was significantly lower; zinc had a transient decrease in absorption; Iron and calcium absorption rates were unchanged	Fei (1993)
Konjac powder	Increasing doses from 50 to 150 mg/kg/day over a nine-day period	Effect of high fiber diets on inorganic ion absorption	Human subjects	No effects of konjac consumption on the absorption of zinc, iron, or calcium in healthy adults	Fei (1993)
Konjac powder	Dietary administration of 10,000 and 20,000 mg/kg/day for 7-8 weeks	Effect of high fiber diets on inorganic ion absorption	Wistar rats	Konjac significantly decreased body weight gain and bone ash levels at 10,000 and 20,000 mg/kg/day. Decreased calcium transport and calcium binding protein was noted in the everted gut sac	Oku *et al.* (1982)
Konjac powder	5000 mg/kg/day for six days	Dietary administration of konjac on lipid profiles	Wistar rats	Konjac significantly decreased plasma and liver cholesterol levels, and decreased the amount of cholesterol in the intestinal wall	Kodama *et al.* (1972)
Konjac powder	500 and 1000 mg/kg acute doses	Oral konjac administration on glucose absorption	Wistar rats	Konjac administration significantly delayed gastric emptying	Ebihara *et al.* (1981b)
Konjac powder	One *percent* solution	Direct perfusion of jejunal segment with konjac on glucose absorption	Wistar rat	Konjac perfusion decreased glucose absorption in the jejunal segment	Ebihara *et al.* (1981b)

Material	Dose/Concentration	Study Objective	Species	Results	Reference
Sodium alginate	Up to 10,000 mg/kg acute dose	Absorption of 14C-labeled sodium alginate	Rat; mouse	The alginic moiety of sodium alginate had only negligible levels of absorption in the gastrointestinal tract	Humphreys and Triffitt (1968); Sharratt and Dearn (1972)
The alginic acid of sodium alginate	2000 mg of alginic acid-based agents *per* 100 ml whey formula	Assessment of calcium iron, or zinc availability from whey-based infant formulas	Human enzyme *in vitro* test system with dialysis bags	Calcium availability was decreased, while iron and zinc availabilities increased.	Bosscher *et al.* (2001)
Sodium alginate	133.3 mg/kg/day for seven days	Absorption of calcium	Human subjects	Sodium alginate did not interfere with calcium absorption	Millis and Reed (1947)
Sodium alginate	Dietary administration at 10,000 mg/kg/day for ten days	Absorption and utilization of protein, fat, and calcium	CD albino rats	Reduction of food intake and weight gain, but no significant effect on protein, lipid, or calcium absorption. One-third of sodium alginate was isolated in the feces, with partial digestion of sodium alginate.	Viola *et al.* (1970)
Sodium alginate	Dietary administration at 2500 mg/kg/day for 28 days	Evaluation of body weight gain	Sprague-Dawley rats	Sodium alginate did not affect overall body weight gain, but	Ohta *et al.* (1997)
Sodium alginate	Acute oral administration at 2500 mg/kg bw	Evaluation of and postprandial glycemic response	Diabetic Sprague-Dawley rats	Sodium alginate reduced the postprandial glycemic response when concurrently fed calcium	Ohta *et al.* (1997)
Sodium alginate	Dietary administration at 3100 and 6300 mg/kg/day for 30 days	Evaluation of magnesium bioavailability during sodium alginate consumption	Wistar rats	Food intake and body weight gain were decreased. Magnesium and calcium levels in the kidneys were significantly decreased, and calcium and magnesium absorption and retention was decreased.	Kikunaga *et al.* (1999)
Xanthan gum	2000 mg/kg/day	Absorption of ^{14}C-labeled xanthan gum	Albino Rats	Xanthan gum at 2000 mg/kg/day resulted in carbon dioxide formation and 98% of radioactivity in feces	Booth *et al.* (1963); JECFA (1987)

w/v = weight/volume

6. SAFETY EVALUATION

6.1. Acute and Short-Term Preclinical Studies

No acute or short-term preclinical studies have been published on PGX. A single-dose oral toxicity study on konjac powder was conducted in CD-1 mice and Sprague-Dawley rats at a maximal dose of 2800 mg/kg (Oketani *et al.*, 1984).[22] No abnormalities or mortalities were noted in the appearance and behavior of either the mice or rats during the 14-day observational period. The LD_{50} for konjac powder is greater than 2800 mg/kg in rats and mice (Oketani *et al.*, 1984). No changes were noted in body weight changes, the status or conditions of feces or anus, occult blood in the urine and feces, and macroscopic findings upon autopsy, compared to controls. Kotkoskie *et al.* (1992) summarized (in abstract form) several acute oral, dermal, and inhalation toxicity tests on konjac. The oral LD_{50} in rats was determined by mixing konjac flour at a dose of 5000 mg/kg with five grams of Purina Rodent Chow 5001, and fed to each animal as the sole source of food for 24 hours. As no mortality was observed during the 14-day post-feeding period, Kotkoskie *et al.* (1992) concluded that the oral LD_{50} was greater than 5000 mg/kg, the highest dose tested. The dermal LD_{50} in rabbits was greater than 2000 mg/kg, the highest dose tested. Unground konjac flour administered to rats in a four-hour acute inhalation study did not result in mortality or adverse clinical signs, with a maximum attainable concentration of 1.5 mg/m^3. No additional details of these studies were available.

Sodium alginate was evaluated for acute toxic effects when injected intraperitoneally in mice at 250, 500 and 1000 mg/kg in three groups of mice (of either sex) (Arora *et al.*, 1968). The mice were observed for any physical signs of toxicity (*e.g.*, sedation, tremors, convulsions, or salivation). Mortality was calculated at 24 and 48 hours post-dose, and post-mortems were performed on all dead mice for any macroscopic evidence of toxic effects. Histopathological studies were performed on the brain, heart, kidney, liver, and spleen. At the end of the 48 hour observation period, 41 out of 50 mice had died in the 1000 mg/kg dose group, while three mice out of ten died at the 500 mg/kg dose. No mortality was observed at the 250 mg dose.

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[22] This dose was judged to be the physiological and physical threshold quantity, based on the viscosity of the konjac powder when dissolved in water (Oketani *et al.*, 1984).

Histopathological examination noted a few nonsignificant hemorrhagic spots in the liver and kidney, but the authors stated that no significant changes were seen. Arora *et al.* (1968) stated that sodium alginate at the same doses in rats did not cause mortality, although data for this statement were not presented. As stated in a summary document of an unpublished report, the oral LD_{50} for sodium alginate in rats is greater than 5000 mg/kg (Woodard Research Corp., 1972). Booth *et al.* (1963) stated an oral LD_{50} for xanthan gum in mice at 1000 mg/kg. In a subacute (30-day) study, sodium alginate was fed to 15 mature male rats at a dietary supplementation of 5% (w/w) (approximately equivalent to 5000 mg/kg/day) for 30 days (Anderson *et al.*, 1991). There was no incidence of diarrhea or loss of normal bowel movement, and none of the standard urinalysis parameters were altered. A full post-mortem examination was conducted on all the rats. Compared to controls, two rats showed mild distension of a portion of the ileum; ten rats showed distension of the cecum, and eight rats showed the colon distended to some degree with soft contents. The formation of fecal pellet did not differ from those of control rats. In another 30-day study summarized by JECFA (Mouecoucou *et al.*, 1990; JECFA, 1993), protein efficiency ratios were not affected in male Wistar rats fed 0, 0.5, 1, 2, or 3% sodium alginate.

In a three-week study evaluating the effects of sodium alginate consumption (20% of the diet, approximately equivalent to 20,000 mg/kg/day) on the absorption and retention of divalent radionuclide cations, male Long-Evans rats (n = 5 *per* group) did not gain weight when compared to controls administered the same amount of diet (20 g of food that did not contain 4 g fiber). When compared to animals administered only 16 g of nutritive diet, body weight differences between control and experimental groups were not significant. Thus, the failure to gain body weight reflects the reduced nutritive intake. Sodium alginate consumption in this study decreased the retention of orally consumed radionuclides (Silva *et al.*, 1970). Van der Borght *et al.* (1971) reported similar radionuclide reductions in mice fed sodium alginate and Kostial *et al.* (1969) previously evaluated sodium alginate fed to suckling and lactating rats and also found that sodium alginate administration decreased strontium absorption.

Sodium alginate was also analyzed for the ability to lower cholesterol by Seal and Mathers (2001), who fed sodium alginate mixed into the diet to male Wistar rats (n = 5 *per*

group) at 0, 5, or 10% (approximately equivalent to 0, 5000 and 10,000 mg/kg bw/day, respectively) for 21 days, which comprised a 14-day adaptation period, followed by a seven-day balance period.[23] During the adaptation period, two rats from the 10,000 mg/kg bw/day dose group were removed due to "large food refusals" (no additional information was provided). However, food refusals were negligible during the balance period and therefore, and dry matter intake (*i.e.*, test substance-diet mixture) was similar across groups. Dry matter digestibility[24] decreased linearly with increasing sodium alginate intake ($P<0.001$). Increased fecal output correlated with increased sodium alginate intake ($P<001$). Total fecal bile acid output rose with a maximum at 5000 mg/kg bw/day sodium alginate, with no further increase at the higher dose ($P<0.05$). Intestinal tissue weight was increased after sodium alginate consumption at both doses ($P<0.001$). In addition, a dose-dependent decrease in plasma cholesterol concentrations was reported ($P<0.05$). The results indicate that sodium alginate has a hypocholesterolemic effect in rats. A study by Kimura *et al.* (1996) found that an acute administration of sodium alginate immediately after a bolus dose of radiolabeled cholesterol enhanced cholesterol excretion ($P<0.05$) into feces. In a separate experiment, sodium alginate administration in combination with glucose inhibited the rise in blood glucose and insulin levels in male Wistar rats, when compared to controls ($P<0.05$) (Kimura *et al.*, 1996).

Xanthan gum has also been evaluated for subacute toxicity, in which adult mice (strain or sex not stated) received, *via* gavage, a total of four doses in water of 1000 mg xanthan gum/kg bodyweight/dose over a period of eight hours (*i.e.*, 4000 mg xanthan gum/kg bodyweight during the study) (Booth *et al.*, 1963). Only 1000 mg/kg xanthan gum could be given *per* dose due to its high level of viscosity. No effect from this treatment was observed. Intraperitoneal injections (0.5 mg xanthan gum in water/injection *per* mouse) into mice were made daily for two five-day periods separated by two days. Abdominal swelling was noted during the injection period, but

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[23] No additional information is provided for the terms "adaptation period" and "balance period". It is inferred in the paper that the animals were dosed for a total 21 days, the first 14 were considered a time during which the rats were "adapting" to the high fiber diet, while in the last seven the rats adapted to the diet and were in "balance" with the fiber intake, and therefore measurements could be initiated.

[24] Dry matter digestibility was calculated by dividing (dry matter consumed minus dry matter in feces) by dry matter consumed (Seal and Mathers, 2001)

there were no deaths during the treatment period and up to two weeks after the final injection. Xanthan gum was not found in the abdominal cavity upon autopsy, from which the authors concluded that xanthan gum was absorbed following injection (Booth *et al.*, 1963). In paired feeding studies, rats ingesting *ad libitum* a diet that contained 7.5% xanthan gum (approximately equivalent to 7500 mg/kg/day) was compared with control rats restricted to the same intake of the basal diet reduced by 7.5%. Weight gains were identical for the restricted and *ad libitum* groups at the end of the 18-day study, in which the authors stated was indicative of the absence of any growth-inhibiting factor associated with xanthan gum. Following a seven-day administration of xanthan gum (0.4 g/rat) *via* the diet to albino rats (strain not stated), caloric availability and digestibility[25] of the gum was calculated, and the authors concluded that there was no net loss or gain in bodyweight observed, and was completely accounted for in the feces, indicating that xanthan gum was not digested and not utilized (Booth *et al.*, 1963). A diet containing xanthan gum at a final fiber concentration of 18,000 mg/kg fed to male Sprague-Dawley rats for two weeks resulted in a thick paste-like mass in the stomach, with a viscosity almost 50 times greater than the viscosity of ingested guar or methylcellulose (Cameron-Smith *et al.*, 1994). Xanthan gum (0, 1000, or 2000 mg/kg bw/day) fed to four groups of two male and two female young adult beagle dogs for two weeks resulted in persistent diarrhea in the high-dose dogs, with a marked reduction in weight. All dogs, including controls, lost weight. The control dogs lost an average of 4% body weight, while the high dose group lost an average of 20% body weight Liver and kidney functions were not altered in this experiment. Extensive gross and histopathological examination did not detect lesions attributable to xanthan gum ingestion (Robbins *et al.*, 1964). Diarrhea and weight loss in dogs fed non-digestible or slowly digested carbohydrate is not uncommon (Burdock and Flamm, 1999).

He and Aoyama (2003) investigated the possibility that dietary fiber consumption could alleviate cystine-induced toxicity in male Wistar rats. The rats (six *per* group) were fed *L*-cystine (35 g cystine/kg diet) with or without konjac (50 g konjac/kg diet) for seven days (konjac consumption at approximately equivalent to 4180 mg/kg bw/day), then evaluated for serum

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[25] Digestibility = (total intake of test material minus increase in fecal weight)/total intake of test material (Booth *et al.*, 1963).

alanine aminotransferase and ornithine carbamoyltransferase activities, as well as serum cholesterol and liver enzyme levels. The addition of cystine to the diet significantly depressed body weight gain, while the addition of konjac to the cystine diet returned body weight gain to basal levels. Cystine consumption significantly increased serum cholesterol ($P<0.05$) and alanine aminotransferase and ornithine carbamoyltransferase activities,[26] and the addition of konjac to the cystine diet decreased these levels to near basal levels. The authors concluded that konjac supplementation significantly reduced cystine-induced liver damage. Administration of 2.5% sodium alginate or 5% konjac in the diet of male Sprague-Dawley rats for four weeks did not promote significant changes in the body weights, compared to untreated controls, and no adverse signs were noted at necropsy (Woo *et al.*, 2007).

In an 18-day study, Hayakawa *et al.* (1999) fed a vitamin B6-deficient diet, with and without 3% of additional konjac (approximately equal to 3000 mg/kg bw/day) to male Wistar rats (n = 5 *per* group) and evaluated the change in vitamin B6 status upon konjac supplementation. Growth rates of the rats lacking vitamin B6 without konjac supplementation were significantly reduced, when compared to vitamin B6- and konjac-containing diets ($P<0.05$) (Table 8). Fecal excretion (*per* day) and fecal content (*per* gram) of vitamin B6 was significantly greater in the konjac-supplemented group, compared to the group lacking vitamin B6 ($P<0.05$). However, there was no significant difference in the fecal excretion of vitamin B6 between the group lacking B6 supplementation and the group that was supplemented with vitamin B6. The authors concluded that konjac was effective for improving the vitamin B6 nutritional state in vitamin B6-deficient rats.

[26] Indicators of liver damage (He and Aoyama, 2003).

Table 8. Body weight, weight gain, food intake and feed efficiency of rats fed a vitamin B6-deficient diet with or without konjac (Hayakawa *et al.*, 1999)

Groups	Initial Body Weight (g)	Final Body Weight (g)	Weight Gain (g)	Food Intake (g)	Feed Efficiency
B6 Deficient	166.8 ± 8.6[1]	192.5 ± 8.6	25.8 ± 2.9[a]	199.6 ± 7.5	0.128 ± 0.011[a]
Konjac (3%) in B6 deficient diet	164.5 ± 7.3	213.1 ± 6.2	48.6 ± 2.1[b]	215.0 ± 2.7	0.224 ± 0.011[b]
B6 Supplemented[2]	163.9 ± 7.4	215.8 ± 6.4	51.9 ± 2.4[b]	214.8 ± 2.1	0.241 ± 0.013[b]

[1]Values are means ± Standard Error for five rats. Means in the same column not sharing the same superscript letter are significantly different at $P<0.05$ according to ANOVA and Duncan's multiple range tests; [2]Pyridoxin-HCI was supplemented at 4.86 μmol/kg diet.

In a nine-week crossover, randomized study, male baboons (n=12) were fed a "Western" diet with or without konjac (5%, approximately equivalent to 1100 mg/kg/day). The effects of these diets on serum and liver lipids, glucose tolerance, insulin response and liver glycogen levels were evaluated (Venter *et al.*, 1990). After nine weeks on the "Western" diet, total serum cholesterol levels were significantly higher than pretest values ($P<0.05$). Ingestion of konjac prevented this increase in cholesterol, and increased HDL cholesterol ($P<0.05$). It also reduced baseline triglyceride values ($P<0.01$) and circulating free fatty acids ($P<0.05$).

Mizutani and Mitsuoka (1987) directly analyzed the effect of a ten-week consumption of dietary konjac (5 and 10%, approximately equivalent to 5000 and 10,000 mg/kg bw/day, respectively) on the composition of fecal microflora and fecal pH in male F344 rats (n = 9 *per* group), when compared to a control diet. Weight gain in the 5000 and 10,000 mg/kg/day dose groups was significantly reduced ($P<0.05$), compared to the control group, as were *Streptococcus* and *Staphylococcus* counts in fecal samples ($P<0.01$). The 10,000 mg/kg/day dose group also had reduced *Clostridium* and *Corynebacterium* fecal counts ($P<0.01$), reduced fecal pH ($P<0.05$), and a significant increase in *Bifidobacterium* fecal bacterial counts ($P<0.01$). This study indicates that konjac consumption alters fecal microflora with a significant increase in *Bifidobacterium* and decreasing potentially pathogenic bacteria.

In summary, although no acute or short-term studies have been conducted on PGX, studies completed on the components of PGX indicate a low potential for toxicity. Acute levels of oral intake of these polysaccharides were limited by their viscous nature, with the oral LD_{50} of konjac or sodium alginate in rats at greater than 5000 mg/kg, and an oral LD50 of 1000 mg/kg for xanthan gum. In short-term studies, sodium alginate at up to 20,000 mg/kg/day for 21 days did not affect body weight, when compared with calorie-restricted controls. Xanthan gum at up to 7500 mg/kg/day for 18 days was well tolerated in rats, with no significant weight loss, compared to feed-restricted controls. Dogs fed 2000 mg/kg/day xanthan gum had persistent diarrhea, although liver, kidney and histopathology evaluations were normal, but this is not an uncommon outcome in dogs fed slowly digestible carbohydrate and should not be compared to rodent or human experience. Konjac has been evaluated in a number of studies, and its consumption in baboons at up to 1100 mg/kg/day for nine weeks or up to 5000 mg/kg/day for four weeks in rats did not alter body weights. At 10,000 mg/kg/day konjac over a ten-week period, rats lost weight, compared to controls. Taken together, these data indicate that consumption of these components of PGX do not cause direct toxicity, but they may cause reduced body weight gain due to reduced caloric intake.

6.2. Subchronic and Chronic Studies

PGX has been evaluated in a 90-day safety study in rats at up to 5% of the diet (Matulka *et al.*, 2008). Sprague-Dawley rats (10/sex/group) consumed 0, 1.25, 2.5, or 5.0% PGX for 90 days, and then were evaluated for toxicological effects. The animals were observed daily for viability, behavioral changes and gross toxicity, with individual food consumption recorded weekly. Mean daily food consumption was calculated for each sex/dietary level at each week. A functional observational battery and a motor activity test were performed on all animals during Week 12 of the study. Clinical pathology and urinalysis parameters were evaluated on test Day

87 for hematology,[27] clinical chemistry[28] and urinalysis[29] and, coagulation on Day 93 (males) and Day 94 (females).

The average daily intake of PGX at 0, 1.25, 2.5, and 5.0% of the diet was determined to be 0, 806, 1617, and 3219 mg/kg/day in males and 0, 918, 1828, and 3799 mg/kg/day in female rats, respectively. Mean feed consumption, body weight, and feed efficiency did not change with PGX consumption (Figure 2 and Figure 3). There were no differences in mean organ weight, organ-to-body weight, or organ-to-brain weight values between controls and treated animals. In male rats fed 5% PGX, a significant decrease in red blood cell count was observed, and increased aspartate and alanine aminotransferase levels and triglyceride levels were reported in female rats, although these effects were not seen in the male rats (Table 9 and Table 10). The aspartate and alanine aminotransferase levels in the female rats were within the historical control value ranges for this laboratory. In female rats fed 5% PGX, significant decreases in serum sodium, potassium and chloride concentrations were observed. In both males and females fed 5% PGX, a significant increase in urinary volume (with a concomitant decrease in urinary specific gravity and total urinary protein) was noted, which may explain the decreased mineral concentrations. These results did not correlate with any histopathological changes. The authors did not consider these effects as adverse, and therefore concluded that the NOAEL for PGX was 5% of the diet, corresponding to an average daily intake of 3219 and 3799 mg/kg bw/day in male and female rats, respectively (Matulka *et al.*, 2008).

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[27] Hematology parameters included erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, absolute reticulocyte count, platelet count, total white blood cell and differential leukocyte count.

[28] Clinical biochemistry values analyzed were: serum aspartate aminotransferase, serum alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, blood urea nitrogen, blood creatinine, total cholesterol, triglycerides, fasting glucose, total serum protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium, and chloride.

[29] Urine was collected from each animal and analyzed for quality, pH, ketone, color, glucose, bilirubin, clarity, specific gravity, blood, volume, protein, urobilinogen and microscopic urine sediment.

Table 9. Mean ± SD[c] of clinical biochemistry values for female rats administered PGX for 90 days

Parameter (units)	% PGX in Diet			
	Control	1.25%	2.5%	5.0%
Aspartate Aminotransferase (U/L)	84 ± 13	86 ± 6	88 ± 10	101 ± 18[a]
Alanine Aminotransferase (U/L)	32 ± 3	38 ± 4	40 ± 7	54 ± 14[a]
Sorbitol Dehydrogenase (U/L)	10.0 ± 2.0	9.2 ± 1.7	10.3 ±2.2	10.4 ± 2.0
Alkaline Phosphatase (U/L)	87 ± 31	97 ± 18	89 ± 25	103 ± 24
Total Bilirubin (mg/dL)	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.02	0.18 ± 0.02
Blood Urea Nitrogen (mg/dL)	18 ± 2	17 ± 2	19 ± 1	18 ± 2
Creatinine (mg/dL)	0.41 ± 0.03	0.42 ± 0.04	0.44 ± 0.03	0.43 ± 0.04
Cholesterol (mg/dL)	98 ± 8	106 ± 11	102 ± 16	111 ± 13
Triglycerides (mg/dL)	25 ± 5	31 ± 8	29 ± 8	34 ± 8[a]
Glucose (mg/dL)	115 ± 8	111 ± 7	115 ± 10	111 ± 7
Total Protein (g/dL)	6.5 ± 0.3	6.5 ± 0.2	6.5 ± 0.3	6.4 ± 0.3
Albumin (g/dL)	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.5 ± 0.2
Globulin (g/dL)	3.0 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	2.9 ± 0.2
Calcium (mg/dL)	10.7 ± 0.4	10.6 ± 0.3	10.5 ± 0.1	10.7 ± 0.4
Inorganic Phosphorus (mg/dL)	5.9 ± 0.5	5.5 ± 0.6	4.9 ± 0.4[b]	5.4 ± 0.7
Sodium (mmol/L)	143.5 ± 5.8	141.0 ± 6.9	143.4 ± 5.6	136.6 ± 5.1[b]
Potassium (mmol/L)	5.02 .± 0.28	4.88 ± 0.25	4.89 ± 0.25	4.71 ± 0.25[b]
Chloride (mmol/L)	104.9 ± 4.0	102.3 ± 5.1	104.8 ± 3.4	100.4 ± 3[b]

[a] Statistically different from control ($P \leq 0.05$) by Dunn's test; [b] Statistically different from control ($P \leq 0.05$) by Dunnett test; [c]SD = standard deviation; dL = decaliters; g = grams; L = liters; mg = milligrams; mmol = millimoles; U = units

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Table 10. Mean ± SD[c] of urinalysis values for male and female rats administered PGX for 90 days

Parameter (units)	% PGX in Diet			
	Control	1.25%	2.5%	5.0%
Males				
Volume (mL)	3.0 ± 1.5	4.0 ± 1.7	6.3 ± 4.6	6.4 ± 3.3[a]
Specific Gravity	1.074 ± 0.02	1.060 ± 0.017	1.054 ± 0.025	1.045 ± 0.018[b]
pH	6.2 ± 0.3	6.4 ± 0.3	6.6 ± 0.3[b]	7.1 ± 0.5[b]
Urobilinogen (EU/dL)	0.6 ± 0.4	0.4 ± 0.4	0.4 ± 0.3	0.5 ± 0.4
Total Protein (mg/dL)	876 ± 683	808 ± 279	745 ± 412	457 ± 346
Females				
Volume (mL)	2.9 ± 3.3	4.0 ± 2.7	1.0 ± 1.0	6.8 ± 2.9[a]
Specific Gravity	1.055 ± 0.026	1.042 ± 0.017	1.070 ± 0.011	1.027 ± 0.008[b]
pH	6.0 ± 0.0	6.2 ± 0.4	6.1 ± 0.2	6.9 ± 0.2[a]
Urobilinogen (EU/dL)	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.4	0.2 ± 0.0
Total Protein (mg/dL)	258 ± 245	196 ± 374	111 ± 66	61 ± 46[a]

[a]$P \leq 0.05$ by Dunn's test; [b]$P \leq 0.05$ by Dunnett/Tamhane-Dunnett test; [c]SD = standard deviation; dL = decaliters; EU = enzyme unit; mg = milligrams; ml = milliliters

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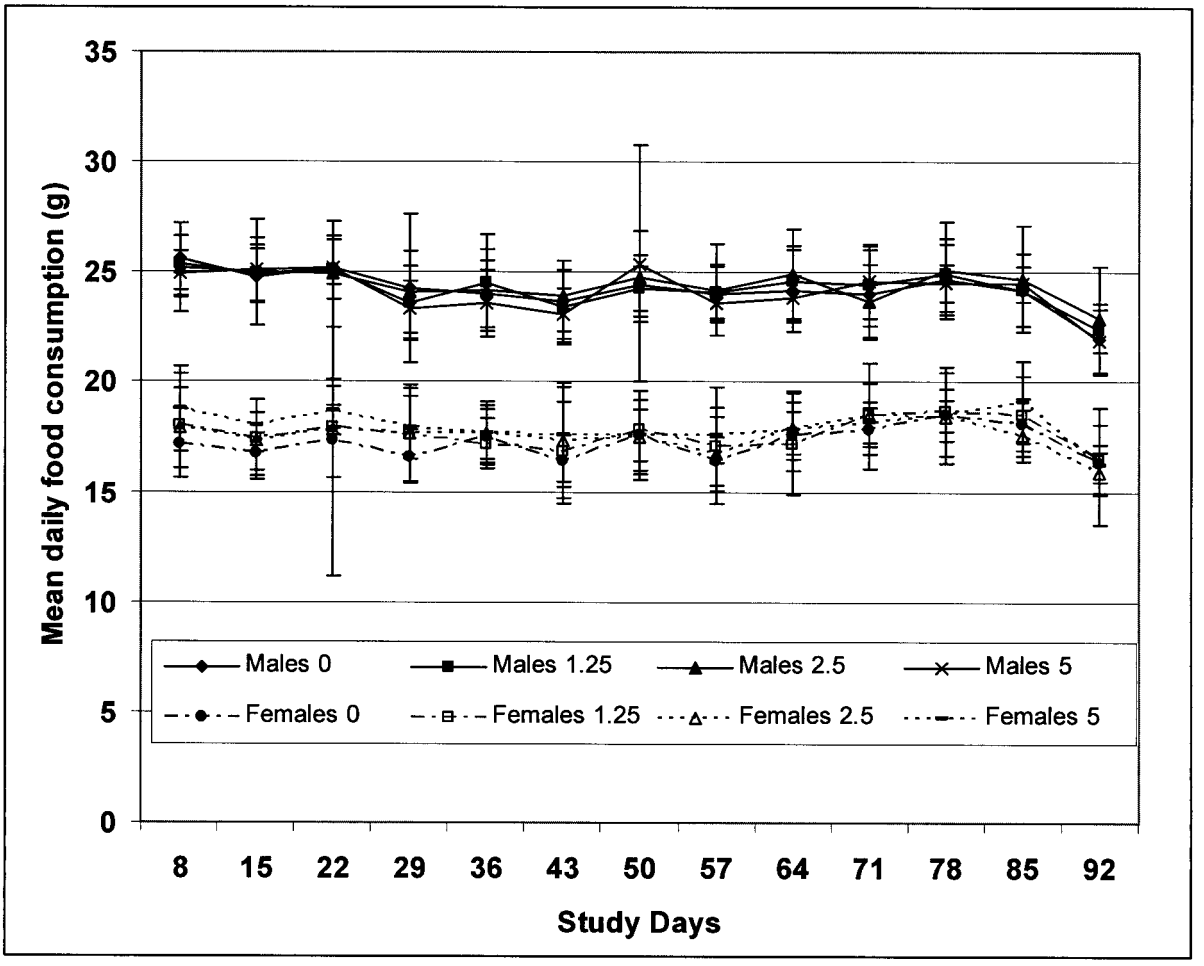

Figure 2. Mean daily food consumption of Sprague-Dawley rats administered PGX in the diet (Matulka *et al.*, 2008)

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No changes were found in neuromotor behavior, as accessed by both fine movements and active movements during a one-hour time period, and no significant changes were noted during the histopathological analysis of the high dose group, as compared to the control group.[30]

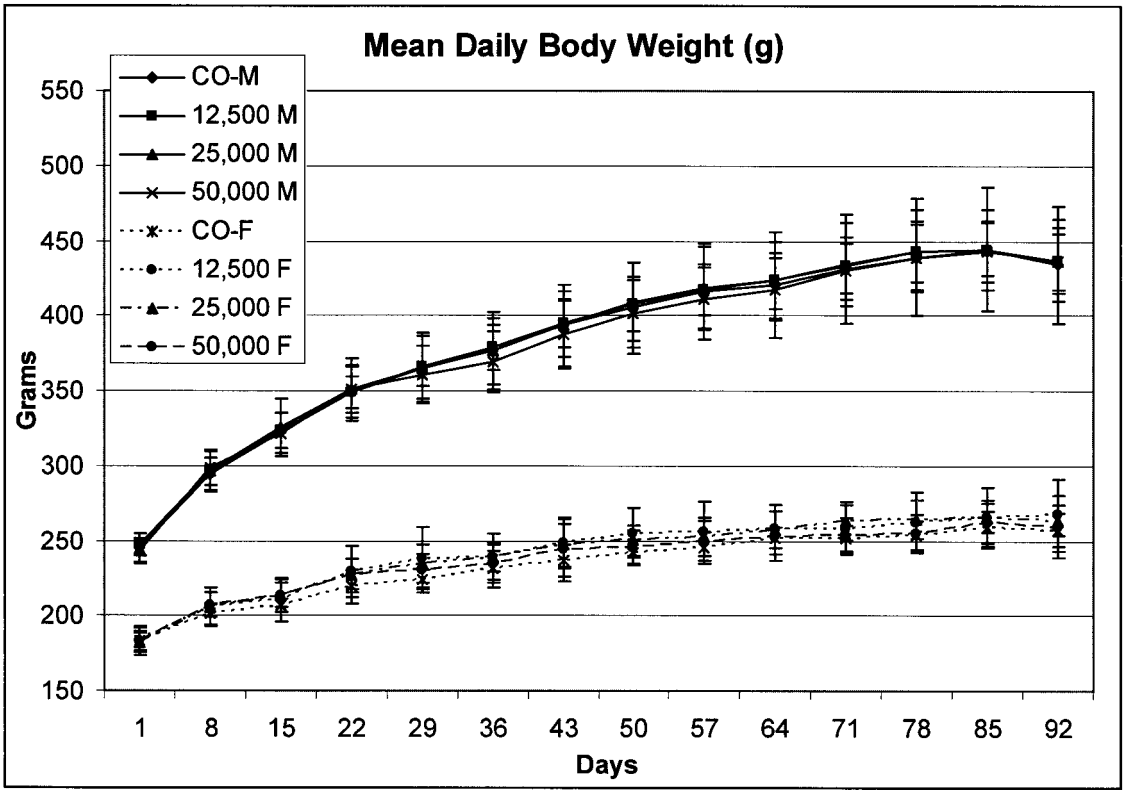

Figure 3. Mean daily body weight gain in Sprague-Dawley rats administered PGX (ppm) in the diet (Matulka *et al.*, 2008)

The individual components of PGX have been evaluated in several preclinical studies. Doi *et al.* (1995) conducted and reviewed several preclinical studies on konjac, and noted that STZ-induced diabetic Sprague-Dawley rats fed konjac at 15% of the diet (approximately

[30] Histological examinations were performed on the preserved organs and tissues from the control and high dietary level groups, which included tissues from the following: adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, female mammary gland, Harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, nasal turbinates, ovaries and testes, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, thymus, thyroid/parathyroid, trachea, urinary bladder, and vagina.

equivalent to 15,000 mg/kg/day) for 18 weeks had significantly reduced blood lipid peroxide levels. Total cholesterol and HDL-cholesterol levels were significantly lower and higher, respectively, in those rats that consumed the konjac diet. Other parameters evaluated (glucose, body weight gain, and triglyceride levels) were not significantly different from controls. Atherosclerotic endpoints indicated that konjac consumption retarded the formation of atherosclerosis in this rat model (Yoshida *et al.*, 1991). In rats fed a hypercholesterolemic diet (control), konjac fed for 12 weeks at 2.5, 5, and 10% of the diet (approximately equivalent to 2500, 5000 and 10,000 mg/kg/day, respectively) was evaluated for effects on elevated serum and liver cholesterol levels (Hou *et al.*, 1990). Consumption of konjac at 10% of the diet returned serum and liver cholesterol levels to those found in a normal basal diet, but did not affect body weight gain, feed intake, or feed efficiency between the 10% group and other groups at the end of the twelfth week of diet administration. During the first eight weeks, the rats fed the 10% konjac had decreased weight gain and decreased feed intake, in which the authors contributed the decreased weight gain to the lower feed intake. Some rats in the 10% konjac group had diarrhea and a poor appetite, but the stools of these animals gradually returned to normal and their feed intake and body weight increased. Administration of konjac did decrease the amount of iron in the stools, when compared to both the basal group and the control hypercholesterolemic group ($P < 0.05$), but the amounts of calcium in the stool were lower than that only in the basal diet group. Stool levels of zinc and copper were not altered by konjac consumption.

Sodium alginate was fed to male albino rats (n = 10 *per* group) at 5.0% of the diet (approximately equivalent to 2500 mg/kg/day) from four weeks of age until 128 weeks of age, with no effect on longevity, compared to controls (Nilson and Wagner, 1951). The alginate groups had similar mean and maximum body weights and consumed similar quantities of food and water, compared to controls. The authors stated that the gross necropsy studies showed "no recurring symptoms which indicated a toxic condition caused by the algin." Earlier ten-week studies at 5, 10, 20, and 30% sodium alginate in the diet (approximately equivalent to 5000, 10,000, 20,000, and 30,000 mg/kg/day, respectively) resulted in food consumption at 3.20, 3.26, 3.48, and 3.87gram *per* gram weight gain, respectively. However, the mean daily body weight gains were 3.81, 3.47, 2.91, and 2.35 g, respectively. The mean water consumed *per* gram gain in

weight was 5.84, 6.28, 8.83, and 13.74 g, respectively. Only two instead of four out of six rats *per* group survived on the two higher levels of sodium alginate. The authors attribute this to the low absorption quality of the basal diet at these exaggerated insoluble fiber consumption levels (Nilson and Wagner, 1951).

A subchronic (*i.e.*, 91-day) study that evaluated only one 15% xanthan gum level (approximately equal to 15,000 mg/kg/day, respectively) in weanling albino rats (strain not stated) reported that xanthan gum at a single dietary level of 15% produced abnormally large fecal pellets, which were well formed, although reduced food intake and a decreased growth rates was observed. In a separate paired-feeding experiment to evaluate the growth rate during xanthan gum consumption, rats that ingested *ad libitum* a diet containing 7.5% xanthan gum had similar body weight gains to control rats fed a basal diet restricted in intake to the amount that the xanthan gum rats consumed (Booth *et al.*, 1963). The authors concluded that this experiment indicated "the absence of any growth-inhibiting factor." In a separate 91-day study, xanthan gum concentrations of 3 and 6% in the diet (approximately equivalent to 3000 and 6000 mg/kg/day, respectively) did not significantly alter weight gains compared to control rats. No significant alterations in organ weights, hemoglobin, or red and white cell counts were observed in rats fed either 3000 or 6000 mg/kg/day xanthan gum for 91 days. No evidence of pathology was noted during histological examination of tissues from rats that ingested diets containing 15% xanthan gum (the only dose of this study). Similar observations were noted in dogs, as stated in a JECFA summary of an unpublished study (JECFA, 1987). Xanthan gum fed to the dogs at 0, 250, or 500 mg/kg bw/day for twelve weeks resulted in softer stools at the high-dose group, but no diarrhea. A nonsignificant retardation of growth and a lower cholesterol level in both sexes of the high-dose group was observed. No additional data were presented (USDA, 1964).

Consumption of xanthan gum administered to albino rats (n = 30/sex/group) *via* the diet for two years at 0, 250, 500, and 1000 mg/kg/day resulted in a lack of significant effect on growth rate, body weight gain, survival, hematologic values, organ weights, or tumor incidence. Survival was comparable between groups, with most deaths occurring after 78 weeks of study. The incidence of spontaneous diseases commonly encountered in aging rats (*e.g.*, respiratory difficulty, soft stools, red rimming around the eyes, unthrifty appearance, labyrinthitis, masses

and open lesions) were comparable for treated and control rats (Woodard *et al.*, 1973). In the same fashion, groups of four male and four female beagle dogs each were fed 0, 250, 370, and 1000 mg/kg/day in the diet for 107 weeks starting at 4-8 months of age (Woodard *et al.*, 1973). Body weights, behavior, blood pressure, electrocardiograms, blood platelet and thrombocyte counts, gross and microscopic examination of tissues, and absolute and relative organ weights did not differ between control and treated groups. Serum alkaline phosphatase, prothrombin time, blood glucose, and serum glutamic-oxaloacetic and pyruvic transaminase were not changed. One 1000 mg/kg male dog had elevated blood urea nitrogen at several test intervals. Stool consistency did not vary significantly between control and treatment groups. The fecal weight increased dose-dependently, and the authors indicated that this weight increase was probably due to the capacity of xanthan gum to take in water. A high-carbohydrate diet containing soluble xanthan gum (250 mg/kg bodyweight/day) was also found to significantly reduce the postprandial glucose response in male Sprague-Dawley rats, compared to similar rats administered a wheat bran-supplemented meal (Cameron-Smith *et al.*, 1994).

In summary, PGX has been evaluated in a subchronic toxicity study in rats, in which a NOAEL was stated at 5% of the diet, the highest dose tested. There were no differences in body weight or organ weight measurements between treated groups and controls, and although several clinical parameters were significantly altered, no histopathological changes were noted. Konjac fed to rats at up to 15,000 mg/kg/day for 18 weeks significantly reduced blood lipid peroxide levels, but no significant reduction in body weight. Sodium alginate fed to rats at approximately 2500 mg/kg/day for over 120 weeks had no effect on longevity, body weights, or food and water consumption, compared to controls. Xanthan gum fed to rats for two years at up to 1000 mg/kg/day did not affect body weight gain, survival, hematologic values, organ weights, or tumor incidence. Feeding of xanthan gum to Beagle dogs at up to 1000 mg/kg/day for 107 weeks did not affect body weight, blood pressure, histopathological analysis of the tissues, or any other clinical parameters, although one high-dose male dog had elevated blood urea nitrogen levels at several test intervals. Overall, literature on PGX or its components indicate a lack of toxicity at the doses evaluated in long-term feeding studies.

6.3. Genotoxicity

PGX has been analyzed for its potential to induce gene mutations in a bacterial reverse mutation test in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, and *Escherichia coli* strain WP2 uvrA (BSL Bioservice, 2008). In two separate experiments, PGX was evaluated at 0, 0.316, 1.00, 3.16, 10.0, 31.6, and 100 µg/plate, with and without metabolic activation *via* phenobarbital-induced Wistar rat liver S9 microsomal fraction. No precipitation of PGX was observed at any of the concentrations utilized. Cytotoxic effects were noted at 100 µg/plate in strain TA1537, with or without metabolic activation. No biologically relevant increases in revertant colony numbers were observed in any of the four *S. typhimurium* strains, or the *E. coli* strain, with or without metabolic activation. Konjac was non-mutagenic in five *S. typhimurium* strains (TA98, TA100, TA1535, TA1537, and TA1538) in the presence or absence of liver microsomal metabolic activation (Kotkoskie *et al.*, 1992). Ishidate *et al.* (1984) reported negative mutagenicity results when sodium alginate was evaluated in the Ames test in *S. typhimurium* with metabolic activation at up to 10 mg/plate, and sodium alginate (10 mg/ml) was negative in a chromosomal aberration study in Chinese hamster lung (CHL) cells. In a dominant lethal assay in ICR/Ha Swiss mice, sodium alginate administered *i.p.*[31] at 82, 200, and 1000 mg/kg bw did not induce genetic toxicity (Epstein *et al.*, 1972). Overall, the published evidence indicates that PGX or its constituents lack genotoxic activity at the concentrations analyzed.

6.4. Carcinogenesis

In a chronic (89-week) study, sodium alginate was fed at 25% of the diet (approximately equivalent to 37,500 mg/kg bw/day) to albino Swiss (SPF) mice and evaluated for potential toxicity and carcinogenicity (Til *et al.*, 1986). To adapt the animals to the high level of sodium alginate, the dietary level was gradually increased for the first 39 weeks of the 89 week study until the maximum achieved dose was 25% of the diet. For each treatment group (n = 75), water intake was measured at Week 87 in at least five animals/sex/group. Hematological examinations were conducted from ten mice/sex/group in Week 40 and 78. Fresh urine samples were obtained

[31] Intraperitoneal

from five males *per* group in Week 82 and from at least eight females *per* group in Week 86. One-half of the surviving male and female mice in each test group were placed on the control diet at Week 87, and after a recovery period of 14 days (Week 89), fresh urine samples were collected for urinalysis. At Week 80, ten mice/sex/group were killed and autopsied. In Weeks 89-92, all survivors were killed and autopsied. Histological examinations were carried out on the kidneys and urinary bladder of all mice, as well as on all organs bearing or suspected of bearing tumors.

The males in the control group and sodium alginate group had an abnormally high mortality rate between Week 39 and 65, and at autopsy approximately 50% of these mice had a hemorrhagic heart with or without hemothorax. This occurrence in sodium alginate-fed mice was not considered to be of toxicological significance due to similar effects in the control group. Urinary incontinence was found in eight males and two females fed sodium alginate, which was not seen in controls. Sodium alginate fed at 25% of the diet was nephrotoxic to mice, as indicated by extremely high water consumption, high urine production, urinary incontinence, high pH and low specific gravity of the urine, increased levels of blood urea nitrogen, increased kidney weights, distension of the renal calyx and the high incidence of dilated distal tubules. Cecal and colonic enlargement and changes in urinalysis were reversible within 2-5 weeks after 87 weeks of treatment (Weeks 89 to 93). In addition, in the female group the sodium alginate diet also increased the incidence of intratubular calcium deposits, and the number of male and female mice with renal pelvic distension and/or distal tubule distension (with increased and enlarged epithelial cells). The increased incidence of intratubular calcium deposits in female mice may have been due to the low occurrence in the control group (13 in the female control group, versus 29 in the male control group). The authors indicated that the high pH and low specific gravity of the urine, extremely high water consumption and urine production, and urinary incontinence were probably due to the high sodium intake in this group. No significant change in the incidence of neoplastic lesions was found in the rats fed sodium alginate. Til *et al.* (1986) concluded that sodium alginate at 25% of the diet (approximately equivalent to 37,500 mg/kg bw/day) in mice for 89 weeks induced pathological changes in the kidney, but did not have any carcinogenic activity.

Mizutani and Mitsuoka (1982) evaluated the effect of konjac on spontaneous liver tumorigenesis and fecal flora in male C3H/He mice. At seven weeks of age, the mice (n = 30/group) were administered a diet containing 10% konjac (approximately equivalent to 15,000 mg/kg bw/day konjac) or a control diet, and maintained on these diets until twelve months of age, at which time they were euthanized and autopsied for the number and size of liver tumor nodules. The weight gain in the konjac-supplemented group was decreased (P<0.05, compared to the control group) throughout the study, although there was no difference in food efficiency between the two groups. The number of liver tumor nodules *per* mouse was significantly lower in the 15,000 mg/kg/day konjac group, while frequency occurrence of bifidobacteria and bacterial counts of enterobacteriaceae were higher in the konjac group, compared to controls (P<0.05). Mizutani and Mitsuoka (1983) went on to evaluate the effect of konjac consumption on 1,2-dimethylhydrazine (DMH)-induced intestinal carcinogenesis in male Fischer 344 rats. Five-week-old rats (n = 20 *per* group) were maintained on a basal diet (control), or the basal diet containing 5% konjac (approximately 5000 mg/kg bw/day) for 20 weeks. Starting at six weeks of age (one week after initiation of konjac consumption), they were administered a weekly *i.p.* injection of 20 mg DMH/kg bw for 13 weeks. Thirteen weeks after the last DMH injection (27 weeks after the start of konjac consumption), the rats were euthanized and autopsied for small and large intestine tumor formation. Konjac administration significantly decreased weight gain (P<0.05), but did not affect feed efficiency, as calculated by the amount of body weight gain divided by food intake at an interval of five weeks. The incidence of DMH-induced colon tumors was significantly reduced with konjac consumption (P<0.05), as well as the number of colon adenocarcinomas *per* animal (P<0.05).

Overall, these studies indicate that the components of PGX are not carcinogenic in nature when fed at high doses, although extremely high doses of sodium alginate have caused pathological changes in the kidney of mice, which may have been attributable to a high salt intake. Konjac did not promote the progression of colonic lesions caused by DMH, but reduced their formation.

6.5. Reproduction Studies

Studies of the effect of PGX on reproductive parameters have not been found in the literature; however, several reproductive studies have been conducted on the constituents of PGX. Xanthan gum was fed at levels of 0, 250, and 500 mg/kg/day in the diet to 10 male and 20 female albino rats of the first generation and 20 rats *per* sex *per* group of the two successive generations in a three generation study (length of time for each generation was not stated) (Woodard *et al.*, 1973). The rats were evaluated for survival, mean body weights, general appearance, behavior and reproductive performance. Females that had fewer than two litters were examined for uterine implantation sites to calculate fetal resorptions. No malformations were found in the offspring. Test and control litters were comparable for all three generations for the number of litters *per* group, numbers of live births, physical condition, and mean weights at birth and weaning, *percent* young alive at weaning and gross autopsy observations. The authors concluded that dietary feeding of xanthan gum at up to 500 mg/kg/day in albino rats had no adverse effect on reproduction.

As stated in a summary of an unpublished study cited in JECFA (1993), rats (20/sex/group) were administered diets containing 0 or 5% sodium alginate (approximately equivalent to 2500 mg/kg/day) for two years. Approximately half of the rats were bred once to produce an F_1 generation, and the F_1 generation was then bred to produce an F_2 generation. It was stated that there were no significant differences in growth rate between the test groups and controls. Reproductive performance, gross and microscopic evaluation of the various tissues and organs, and hematological values were normal. No additional data was provided (Morgan, undated).

In summary, although no reproduction studies have been conducted on PGX, reproduction studies on xanthan gum at up to 500 mg/kg/day and sodium alginate at 2500 mg/kg/day in rats did not reveal adverse effects.

6.6. Other Studies

A five *percent* aqueous solution of xanthan gum topically applied daily to shaved areas of rabbit skin caused localized irritation followed by skin cracking and bleeding; however the

application of water-soluble cornstarch solutions produced similar effects. The authors contributed the dermal effects to continuous moistening and drying of the skin, as rapid healing occurred when the applications were discontinued. In contrast, dietary administration of pulverized konjac powder at 5% of the diet consumed for eight weeks (approximately 7500 mg/kg/day) prevented the development of allergic rhinitis-like inflammation and IgE responses in BALB/c mice treated with ovalbumin (*i.e.*, an initial *i.p.* immunization, then an intranasal challenge with ovalbumin) (Onishi *et al.*, 2007).

Konjac has been utilized as an inhaled sensitizer for the determination of cyclic guanosine 3`, 5`-monophosphate (cGMP) and cyclic adenosine 3`, 5`-monophosphate (cAMP) levels in the nasal mucosa, trachea, lung and plasma of a guinea pig model (Banno, 1979). Male Hartley guinea pigs that were placed in inhalation boxes inhaled konjac powder for 30 minutes once daily for ten days (konjac concentration not stated). Three weeks after the sensitization period, the guinea pigs were again exposed to konjac powder, which induced an asthma-like paroxysm. Approximately 3-10 minutes after the state of paroxysm became stabilized, the animals were ensanguined and tissues and plasma were collected for cGMP and cAMP determination. Based on the measured nucleotide levels in the nasal mucosa, trachea, and lung, Banno concluded that konjac powder-induced sensitization and experimentally-induced asthma in guinea pigs. cGMP was significantly increased in the trachea, and plasma, and cGMP and cAMP levels were significantly increased in the lung during konjac powder challenge.

Overall, this information indicates that dermal or oral administration of the components of PGX has a low potential to produce allergic reactions. Inhalation of konjac may cause pulmonary irritation, resulting in increased inflammatory reactions.

6.7. Observations in Humans

A 21-day randomized, double-blind, placebo-controlled study was conducted on PGX to evaluate the tolerance to ingestion of PGX at 5000 mg *per* day (for the first seven days) and 10,000 mg *per* day (for the last 14 days) (167 mg/kg bw/day in a 60 kg person) in healthy male and female subjects (n = 54) (Carabin *et al.*, 2008). No differences were noted between test and control groups on averaged flatulence *per* day, or average intensity of borborygmi and bloating

per day, vomiting or number of stools. There were no serious adverse events reported during the study. The adverse events were of mild to moderate intensity. One severe episode of headache occurred in one test subject, but the authors considered this event unrelated to product intake. Of the total number of adverse events (21), 14 concerned GI signs and symptoms. Nine of the adverse events were considered unrelated to PGX administration[32], while the adverse events considered as "possibly" related to PGX consumption were one episode each of flatulence in the control group and the study group, and one episode of nausea in the test group. The authors concluded that PGX was "well tolerated by healthy male and female subjects when used to supplement the diet for up to 10,000 mg *per* day."

Sodium alginate has been evaluated for adverse effects in subjects (n = 5 males, average body weight at 76.1 kg) when consumed at 175 mg/kg body weight/day for seven days (13,317 mg/day), followed by 200 mg/kg body weight/day (15,220 mg/day) for an additional 16 days (Anderson *et al.*, 1991). Sodium alginate was consumed three times *per* day, with the fully hydrated sodium alginate added to a pre-determined amount of orange juice, immediately prior to consumption. Diet diaries were maintained by all volunteers during the control and dietary supplementation weeks. Sodium alginate acted as a fecal bulking agent in all volunteers, and resulted in a significant (P<0.01) increase in fecal daily wet weight, and also increased fecal water content and daily dry weight. Fecal pH was not altered. Dietary transit time was not significantly changed during sodium alginate consumption, and had no significant effects on blood glucose and plasma insulin concentrations, breath hydrogen concentrations, hematological indices, plasma biochemistry parameters, and urinalysis parameters. None of the volunteers reported any allergic responses, gastrointestinal disturbances, feelings of distension or abdominal discomfort, or unusual looseness of bowel during sodium alginate supplementation (Anderson *et al.*, 1991). In the same year, Torsdottir *et al.* (1991) reported that consumption of a meal containing 5000 mg sodium alginate by non-insulin-dependent (type 2) diabetics, significantly reduced the postprandial rise in blood glucose, serum insulin and plasma C-peptide concentrations (P<0.05), and increased the gastric emptying half-time, compared to the control

[32] One episode each of pharyngitis, food poisoning, rhinopharyngitis, neutropenia, and three episodes of headache (for one subject) in the control group, and one episode of headache and one episode of soft stool in the study group.

meal. Recent randomized, placebo-controlled, two-way crossover studies found that sodium alginate consumption (1500 mg/day), reduced cholesterol and glucose uptake in overweight male subjects when taken as a single dose, and reduced mean energy intake ($P<0.05$) in males and females (n = 68 males and females; BMI range: 18.50-32.81 kg/m^2) when consumed over a seven-day period. No side effects were reported during the trial (Paxman *et al.*, 2008a; 2008b). In ileostomy subjects (n = 6), sodium alginate (7500 mg/day for four days) increased fat excretion by 140% ($P<0.05$) and decreased bile acid excretion ($P<0.05$), but did not alter the absorption of phosphorus, calcium magnesium, zinc, iron, or manganese (Sandberg *et al.*, 1994). Sodium alginate (10,000 mg/day for two weeks) consumed by eight male subjects increased fecal levels of bifidobacteria ($P<0.05$), while the levels of Enterobacteriaceae and the frequency of lecithinase-negative clostridia occurrence tended to decrease ($P>0.05$). Fecal ammonia and skatole concentrations were reduced ($P<0.05$), and excretion of acetic and propionic acid was increased ($P<0.05$) (Terada *et al.*, 1995).

Alginic acid at 15,000 mg *per* serving three times *per* day (45,000 mg/day) for seven days was provided to three patients whose clinical condition required sodium restriction and one control subject. Significant increases in the daily average excretion of fecal sodium ($P<0.05$) and potassium ($P<0.01$) occurred in two of the three patients, but did not significantly increase daily stool weights. Urinary sodium and potassium levels were not affected. The authors reported that the alginic acid was well tolerated at 45,000 mg *per* day, and was only mildly laxative in nature (Feldman *et al.*, 1952). The lack of reductions in fecal sodium and potassium levels by consumption of sodium alginate suggest that the alginic acid is already buffered with sodium, and therefore would not bind additional sodium from the system.

Konjac has been analyzed for various effects in humans. A study by Huang *et al.* (1990) reported that konjac, consumed at an average of 8600 mg/day for 65 days in a clinical trial containing 72 type II diabetic subjects, resulted in significant reductions in the fasting blood glucose and 2-hour postprandial blood glucose levels after food ingestion ($P\leq0.01$), as well as significant reductions in triglyceride values in those subjects with hypertriglyceridemia (>200 mg%). Konjac intake did not have a significant effect on blood lipid values. Of the 72 type II diabetic subjects, 69-90% reported improved appetite, polyuria at night, thirst, and soft stools or

constipation. Approximately 55% of the subjects reported symptoms such as loose stools, flatulence, diarrhea, and abdominal pain, sounds, or distention.

An earlier study evaluated konjac intake (45 days) in a clinical trial with a total of 110 elderly subjects with hyperlipidemia (Zhang *et al.*, 1990). The experimental group (n=66) consumed an ordinary diet plus 5-10 g/day konjac,[33] and the control group (n = 44) consumed an ordinary diet lacking konjac. The subjects initially consumed three grams *per* day for 2-3 days, after which the full amount was administered. The subjects were evaluated for effects of konjac consumption on triglyceride blood levels (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Compared to controls, consumption of konjac significantly lowered blood levels of TG, TC, and LDL-C (P<0.01), and elevated HDL-C levels. The authors indicated that 17 subjects excreted more bulk feces (compared to controls), three subjects experienced diarrhea, and four subjects stated that they felt hungry more often. In addition, 27 subjects (40.9% of the total) lost body weight during consumption of konjac (P<0.01), and in 13 subjects chronic constipation was relieved. To this end, Loening-Baucke *et al.* (2004) evaluated the effects of konjac in children (4.5 to 11.7 years of age) with chronic constipation in a double-blind, randomized, crossover study. All of the 31 children (16 boys and 15 girls) had functional constipation (*i.e.*, constipation not attributable to organic and anatomic causes or intake of medication), and 18 had encopresis[34] at enrollment. Konjac consumed at 100 mg/kg bw/day (maximal of 5 g/day) for four weeks resulted in significantly fewer children that complained of abdominal pain (P<0.05), while the parents rated significantly more children (68%) as being better when consuming konjac (P<0.001), as compared to positive effects while on the placebo (13%). No significant side effects such as abdominal distention, anaphylactic symptoms, bloating, diarrhea, excessive gas, or new onset of abdominal pain were reported during the study (Loening-Baucke *et al.*, 2004).

[33] Five grams of the refined konjac (in various edible forms) was consumed *per* person once or twice *per* day, but the reason for the large variation in consumption was not stated by the authors (Mao-Yu *et al.*, 1990).

[34] Encopresis= involuntary loss of formed, semi-formed, or liquid stool into the child's underwear in the presence of functional constipation after the child had reached four years of age (Loening-Baucke *et al.*, 2004).

Vuksan *et al.* (1999) found that konjac consumption (a range of 24,000 – 50,000 mg/day, according to energy intake)[35] (consumption was 0.7 g konjac *per* 412 kJ [100 kcal] of energy) significantly reduced serum fructosamine ($P<0.01$), the total:HDL cholesterol ratio ($P<0.05$), and systolic blood pressure ($P<0.05$), compared to the placebo, in a randomized, controlled metabolic trial containing two three-week phases separated by a two-week washout period in eleven hyperlipidemic and hypertensive type 2 diabetic patients. Body weight, diastolic blood pressure, total LDL and HDL cholesterol, triglycerides, apolipoproteins A-1, B, and their ratio, glucose, and insulin levels were not affected. A transient complaint of flatulence and soft stools was reported by 37 and 24% of the subjects during the konjac and placebo control treatments, respectively. None of the subjects refused to continue the study due to these complaints. In a similar study, Vuksan *et al.* (2000) reported that a dietary intake of 8000 – 13,000 mg/day konjac improved glycemic control and the lipid profile in subjects with impaired glucose tolerance, reduced HDL cholesterol, elevated serum triglycerides, and moderate hypertension. These studies extended earlier work by Renard *et al.*, (1991), who found that konjac consumption (3000 mg/day) decreased mean postprandial serum glucose and insulin increases after a carbohydrate-rich meal. Azezli *et al.* (2007) also found that patients being treated with propanolol for hyperthyroidism benefited from consumption of glucomannan at 2600 mg/day, during a two-month treatment period. Significantly lower serum T3, T4, FT3 and FT4 levels ($P<0.05$) were reported.[36]

Ebihara *et al.* (1981a) evaluated the effects of konjac administration on plasma glucose and insulin responses to orally administered glucose consumption in men (n = 7). In this crossover study, groups of three and four subjects drank a control meal (500 ml of a 16% glucose solution, resulting in 80 g of glucose consumed) or a test meal (500 ml of the 16% glucose solution) which also contained 5000 mg of konjac (approximately equivalent to 83.3 mg/kg konjac). Plasma glucose and insulin concentrations were determined at 30, 60, 90, 120, and 180 minutes after meal consumption. Consumption of konjac decreased the peak plasma glucose

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[35] Total dietary fiber, which included konjac, was 2 g/412 kJ (100 kcal) *per* day (Vuksan *et al.*, 1999)

[36] T3 = triiodothyronine; T4 = thyroxine; FT3 = free T3; FT4 = free T4

concentration noted at 30 minutes post-dose and reduced the area under the curve, when compared to the control meal, but not significantly. Plasma glucose concentrations following combined glucose and konjac consumption were consistently lower during 120 minutes post-consumption, compared to glucose consumption alone, although at 180 minutes post-dose, the glucose level in the konjac-dosed men was significantly higher than the controls ($P<0.05$). The insulin response following konjac and glucose ingestion was significantly lower than glucose ingestion alone ($P<0.05$), and remained lower until the 180-minute time period. These data indicate that konjac consumption attenuated a sharp increase in plasma glucose and insulin levels following glucose ingestion. Morgan *et al.* (1990) found that an acute dose of konjac (5000 mg) lowered post-prandial insulin levels, but did not affect circulating gastric inhibitory polypeptide levels.

Arvill and Bodin (1995) examined the hypocholesterolemic effect of supplementing the diet of normocholesterolemic or hypercholesterolemic, but otherwise healthy men ($n = 63$) with konjac. After a two-week baseline period, the subjects were given 3870 mg konjac or placebo *per* day (three capsules taken three times daily one-half hour before meals, each capsule containing 430 mg konjac) for four weeks in a double-blind crossover, placebo-controlled study. Konjac reduced total cholesterol ($P<0.0001$), low-density-lipoprotein cholesterol (LDL-C; $P<0.007$), triglycerides ($P<0.03$), and systolic blood pressure ($P<0.02$). High-density lipoprotein cholesterol (HDL-C) and the ratio of LDL-C to HDL-C were not significantly altered. No changes in diastolic blood pressure or body weight were observed. No adverse effects were observed. This follows an earlier study by Terasawa *et al.* (1979) conducted in elderly women (older than 70 years; $n = 12$) who consumed 3000 mg/day konjac (approximately equivalent to 66.67 mg/kg/day in the 45 kg women) for four weeks. This consumption resulted in significant reductions in serum cholesterol and triglyceride values ($P<0.05$), but no change in body weights. The authors noted that no allergenic or gastrointestinal disturbances were observed in any of the subjects. The above studies indicate that konjac consumption at approximately 3000 mg/day (1000 mg/meal) is without adverse effects and may lower LDL-C concentration and LDL-C/HDL-C ratios, while maintaining body weight. In an earlier double-blind crossover study, in which overweight patients with osteoarthrosis ($n = 20$) consumed 3000 mg konjac (1500 mg just

before the two principal meals) *per* day for eight weeks, a significant ($P<0.02$) reduction in mean body weight of 3.7 kg was observed (Biancardi *et al.*, 1989). The consumption of konjac was well tolerated, with only one patient dropping out of the trial because of abdominal discomfort during the konjac treatment. No changes in blood pressure, heart rate, or biochemistry (exact parameters measured were not stated) were noted after konjac treatment. These results are in agreement with work by Birketvedt *et al.* (2005), who reported that overweight subjects ($n = 53$ males and females) that consumed 4320 mg/day of a konjac supplement *per* day for five weeks during a randomized placebo-controlled study lost a significant amount of weight ($P<0.001$). However, in subjects already losing weight ($n = 30$), Wood *et al.* (2007) reported that additional soluble fiber from konjac (3000 mg/day) did not increase weight loss.

The Arvill and Bodin (1995) work follows a study conducted by Matsuura (1986) that examined the effects of konjac consumption on serum and fecal bile acids, gastrointestinal transit time (GTT), fecal weight and exocrine pancreas function in normal subjects. As described in a summary of this study published in Japanese, eight subjects were given 7800 mg konjac daily for three weeks, with a control observation period of one week prior to and after the konjac consumption. Fecal weight increased during the konjac period, as compared to the control period (control at 149.7±16.5 g/day *vs.* 181.4±26.0 g/day during the konjac period). The majority of the increase in fecal weight was due to fecal water, rather than fecal solids. The GTT was accelerated during konjac consumption ($P<0.01$), as well as the fecal excretion of fat ($P<0.05$), although the fecal excretion of nitrogen and protein did not change. Fasting serum bile acid levels were significantly reduced during the konjac consumption period, with an increase of the fecal excretion of bile acids. Serum cholesterol levels were nonsignificantly reduced during konjac consumption, compared to controls (158.4±16.5 *vs.* 183.1±15.2 mg/dL, respectively). Overall, there was no change in mean body weight and stool frequency observed during the study. This study indicates that konjac binds bile acids and cholesterol in the small intestine and inhibits their reabsorption.

In a randomized double-blind study to evaluate the effects of konjac consumption (3600 mg/day for 28 days) on blood lipid and glucose levels in hyperlipidemic type 2 diabetic patients (n=22), Chen *et al.* (2003) found that konjac reduced plasma cholesterol, LDL-cholesterol,

total/HDL cholesterol ratio, ApoB, and fasting glucose (all $P<0.01$), when compared to placebo (corn starch). Body weight, plasma triglyceride, HDL-cholesterol, LDL/HDL cholesterol, and postprandial glucose levels were not significantly altered after konjac consumption. Fecal neutral sterols and bile acid concentrations were increased by 18% and 75%, respectively ($P<0.01$). The experimental protocol was followed carefully by the patients (95% of the konjac was consumed during the study). One subject developed minor gastric discomfort during the start of the study, but adapted by the fifth day of the konjac supplementation. Results of this study indicate that konjac at 3600 mg/day is well tolerated, and blood lipid levels (except triglyceride) were improved during the konjac consumption period.

Walsh *et al.* (1984) utilized an eight-week double-blind trial to evaluate the effects of administration of 1000 mg doses of konjac one hour prior to each of three meals *per* day (3000 mg/day), and found that a significant mean weight loss occurred over the eight week period. Serum cholesterol and LDL cholesterol were significantly reduced ($P<0.05$), compared to controls. No adverse reactions to konjac were reported.

Konjac has also been examined for its laxative effects in a placebo-controlled study that consisted of a 21-day placebo period, a seven-day adaptation period when the subjects ($n = 8$; one man and seven women) consumed progressively larger amounts of konjac (1500 to 3000 mg/day), and a 21-day konjac-supplemented period (1500 mg/meal; 4500 mg/day) (Chen *et al.*, 2006). The gastrointestinal response was monitored daily, and stool samples were collected on days 15-21 of placebo (*i.e.*, corn-starch) and konjac-supplemented periods, to determine short-chain fatty acid contents, fecal mass, components, and microflora. Results of this study showed that konjac supplementation significantly increased the mean frequency of defecation by approximately 27% ($P<0.05$), eased the passage of bowel movement ($P<0.05$), and slightly improved ($P<0.01$) the completeness of relief after defecation (Table 11). Konjac supplementation did not increase abdominal cramping, borborygmi, bloating, or flatulence. The stool consistency was not significantly different between groups. Konjac administration also increased fecal weight and moisture output (Table 11), with the increase in dry mass mainly contributed to increases in the fecal plant and soluble fractions ($P<0.05$). Elevated concentrations of fecal lactobacilli, total bacteria, and daily fecal output of bifidobacteria ($P<0.05$), lactobacilli

(P<0.05), and total bacteria (P<0.05) were also noted after konjac consumption (Table 11) (Chen *et al.*, 2006).

Table 11. Summary of gastrointestinal responses and colonic ecology after konjac consumption (Chen *et al.*, 2006)

	Placebo	Konjac (4.5 g/day)
Gastrointestinal response on Days 15-21 of placebo and konjac consumption		
Defecation (number/day)	1.1 ± 0.2	1.4 ± 0.2*
Ease of bowel movement (*per* defecation)	2.0 ± 0.2	1.7 ± 0.2*
Abdominal cramping (*per* day)	0.3 ± 0.2	0.3 ± 0.1
Borborygmi (*per* day)	0.1 ± 0.1	0.0 ± 0.0
Bloating (*per* day)	0.2 ± 0.1	0.1 ± 0.1
Flatulence (*per* day)	1.2 ± 0.2	1.3 ± 0.3
Stool consistency (*per* stool)	3.0 ± 0.1	3.1 ± 0.2
Fecal weight		
Wet weight (g/day)	132.0 ± 26.5	171.8 ± 29.3
Dry weight (g/day)	28.6 ± 4.0	34.8 ± 5.7*
Fractions (g/day)		
Plant	4.1 ± 0.6	5.8 ± 0.6*
Bacterial	12.9 ± 1.6	13.6 ± 2.7
Soluble	9.5 ± 2.1	12.9 ± 2.4*
Moisture (g/day)	103.2 ± 22.7	136.7 ± 23.7*
% Total fecal microflora		
Bifidobacterium	8.3 ± 1.6	15.4 ± 2.7*
Lactobacillus	2.8 ± 0.5	4.9 ± 0.8*
Bacteroides	3.1 ± 0.8	3.8 ± 0.5
Clostridium	15.3 ± 2.2	10.1 ± 1.8*

*P<0.05; Values given are means ± standard error measurements.

Doi *et al.* (1982) evaluated the effects of high-viscosity konjac on glucose and lipid metabolism in normal (n = 24) and diabetic (n = 21) subjects. When compared to controls, the mean blood glucose levels of the subjects receiving konjac (3900 mg in powdered form) were significantly reduced at 90 minutes post-dose (P<0.05). When administered to healthy subjects 15 minutes prior to a test meal, the mean blood glucose levels were significantly reduced below controls at 30 and 180 minutes, but the mean insulin levels were not significantly reduced. When konjac was mixed into the test meals, the mean blood glucose levels were significantly reduced below control levels at 30, 60, 120, and 180 minutes. Serum insulin levels were also significantly

below control levels at 30, 60, and 90 minutes post-dose. When administered to six non-insulin-dependent diabetics, the mean blood glucose levels were below control subjects at 30 and 60 minutes post-dose (Doi *et al.*, 1982). When konjac was added to test meals and given to nine healthy subjects, no significant increase in the mean postprandial blood glucose levels was seen during the observation period.

In continuing the evaluation of possible glucose malabsorption during konjac consumption, a xylose absorption test was performed on five healthy subjects to check for malabsorption. A significant decrease in two-hour urinary xylose excretion was observed with 3000 mg of konjac ($P<0.05$). However, over a six-hour period, the total excretion of xylose was the same in both control and konjac-treated subjects. The authors concluded that konjac reduces the postprandial rise of glucose levels by the same mechanism as guar gum (Doi *et al.*, 1982). Supplementation of the diet with 7200 mg of konjac for 17 days to 21 diabetic patients resulted in significant reductions in the mean fasting blood glucose over the 17-day period ($P<0.05$). The mean serum cholesterol level was observed for 90 days, and fell significantly for the first 38 days ($P<0.05$), then gradually rose, while triglyceride and HDL-cholesterol levels were not affected (Doi *et al.*, 1982). Doi *et al.* (1983) also reported that konjac consumption (an acute ingestion of 3900 mg konjac with a meal) reduced vitamin E absorption into the intestine ($P<0.05$), while vitamin B12 was not reduced in eleven subjects (six normal and five maturity onset diabetics). The authors indicated that konjac may inhibit fat-soluble, but not fat-insoluble, vitamin absorption through the removal of bile acids from the system.

Eastwood *et al.* (1987) evaluated the effects of xanthan gum consumption on dietary, biochemical, hematological, fecal, and physiological changes in healthy men ($n = 5$). The men consumed 150 mg/kg/day xanthan gum (approximately 10,000 – 12,000 mg/day, in three divided doses) for a 23-day treatment period. Xanthan ingestion at 150 mg/kg/day for 23 days had no significant effect on plasma biochemistry, glucose and insulin tests, hematological indices, serum immunoglobulins, urinalysis parameters, HDL cholesterol, phospholipids, triglycerides, or on breath hydrogen and breath methane concentrations. Xanthan gum ingestion did reduce ($P<0.05$) serum cholesterol, and significantly increased fecal bile acid concentrations ($P<0.05$). Xanthan gum also acted as a bulking agent, increasing fecal dry and wet weight, and decreased the

average transit time from 56 to 45 hours. Xanthan gum consumption (15,000 mg/day) for ten days by 18 subjects also caused significant increases in stool output ($P<0.01$) frequency of defecation ($P<0.05$), and flatulence ($P<0.01$), but did not significantly effect transit time (Daly *et al.*, 1993). Daly *et al.* (1993) also evaluated the possibility of bacterial adaptation during xanthan gum consumption, (as determined by the ability of fecal samples to reduce the viscosity of xanthan gum *in vitro*), and reported that before xanthan gum consumption, fecal samples from 12 of the 18 subjects could reduce the viscosity of the gum *in vitro*, and this number increased to 16 subjects after xanthan gum consumption. *In vitro* production of hydrogen and short-chain fatty acids also increased after xanthan gum consumption ($P<0.05$).

To summarize, studies conducted on PGX or its components indicate that PGX is well tolerated when consumed at 10,000 mg/day (167 mg/kg bw/day in a 60 kg person) for 21 days, the highest dose tested. The individual components of PGX have been consumed in clinical trials, with sodium alginate consumed for 16 days at 15,220 mg/day (200 mg/kg bw/day) resulting in increased fecal daily wet weight, water content and dry weight. Other studies on sodium alginate at up to 10,000 mg/day for two weeks positively altered microbial populations in fecal samples, while sodium alginate at 7500 mg/day for four days increased fat excretion, but did not alter mineral absorption. Konjac has not resulted in adverse effects, when consumed at up to 10,000 mg/day for up to 45 days. At up to 50,000 mg/day konjac (approximately equivalent to 833 mg/kg/day), the only adverse events noted were transient complaints of flatulence and soft stools. No adverse effects were noted on measured parameters, including body weight, blood pressure, cholesterol, glucose, or insulin levels. Xanthan ingestion at 150 mg/kg/day for 23 days in male subjects (equivalent to a maximum of 12,000 mg/day) had no significant effect on any parameters evaluated, except a significant decrease in serum cholesterol, and a significant increase in fecal bile acid. Overall, these studies indicate that PGX and its components are well tolerated by humans at the doses evaluated, with a low potential for intolerance at extremely high fiber doses. These events are not considered toxic, and the consumer would be able to modulate the amount of fiber consumption to reduce the incidence of these events.

7. EVALUATION

PGX is an agglomeration of the three soluble fibers: konjac glucomannan, sodium alginate, and xanthan gum, which results in a new ingredient that has three to five times greater viscosity than any other soluble fiber. PGX will be used as an ingredient to provide a source of fiber in the diet. PGX is not regulated by FDA, but the components konjac, sodium alginate, and xanthan gum have been determined GRAS for various uses. PGX is manufactured by initially blending the three ingredients, then processed through agglomeration and drying to form the final novel product.

Fiber is considered a nutrient, with a recommendation of up to 25 g fiber consumption *per* day as part of a healthy diet in women, and up to 38 g fiber *per* day for men. Konjac, sodium alginate, and xanthan gum are soluble fibers that are not metabolized in the small intestine, but may be metabolized to a small extent by gut microflora to SCFA and carbon dioxide. Several studies have been conducted on PGX, and many have been conducted on the single ingredients. PGX has been evaluated in a 91-day toxicity study in rats, in which a NOAEL was stated at 5% of the diet, equivalent to 3799 mg/kg/day, the highest dose tested. The components of PGX have been evaluated in a variety of preclinical and clinical studies, with any adverse effects generally related to those symptoms related to extremely high fiber intake. A clinical study found that PGX is well tolerated at 10,000 mg/day for 21 days, while clinical trials on the components have found no adverse effects at 10,000 mg/day or greater levels for duration of up to 45 days, other than possible intolerance to high levels of fiber, indicated by diarrhea, flatulence, or discomfort.

The NOAEL for PGX in rats is 3799 mg/kg/day (equivalent to 227,940 mg/day in a 60 kg human) and a tolerable intake of PGX in humans identified is at least 10,000 mg/day, the highest dose administered. Combining the 90th percentile PGX consumption levels from foods supplemented with PGX (10,070 mg/day) with the potential PGX consumption from dietary supplements which may add an additional maximum intake of 3000 mg/day[37], results in the potential theoretical maximum PGX consumption that may reach 13,070 mg/day (217.8

[37] This estimate is based on current labeled information rather than on a statistical analysis of consumption because consumption data are unavailable.

mg/kg/day for a 60 kg person). This theoretical intake level represents a conservative estimate because it is unlikely that an individual would consume PGX from both conventional foods at the 90th percentile and dietary supplements, and is still within the recommended daily intake of fiber. Thus, based on the generally available data establishing the PGX NOAEL at 3799 mg/kg/day in female rats, the highest dose administered, the Panel has determined a safe intake of PGX at 217.8 mg/kg/day or 13,070 mg/day for a 60 kg individual.

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8. CERTIFICATION

The undersigned authors of this document—a dossier in support of GRAS status determination for use of PGX—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of the available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

08JAN09

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9. CONCLUSION

After critically evaluating the information available, the Expert Panel has determined that, based on common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, there is reasonable certainty that PGX, produced in accordance with current Good Manufacturing Practice (cGMP), is safe under the intended conditions of use, and is therefore Generally Recognized As Safe (GRAS), by scientific procedures, when used as an ingredient to add fiber to the diet, such that total daily consumption of PGX from all sources will be 13,070 mg PGX. In particular, the Expert Panel has evaluated the proposed use of PGX at specified levels in the foods listed in Table 5 of this document and has concluded that such use is Generally Recognized As Safe (GRAS).

10. SIGNATURES

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APPENDIX A

Table A. Foods for the addition of PGX

Description	Ingredient (mg/g)
YOGURT, NS AS TO TYPE OF MILK/FLAVOR	11.11111
YOGURT, PLAIN, NS AS TO TYPE OF MILK	11.11111
YOGURT, PLAIN, WHOLE MILK	11.11111
YOGURT, PLAIN, LOWFAT MILK	11.11111
YOGURT, PLAIN, NONFAT MILK	11.11111
YOGURT, VANILLA, LEMON, COFFEE, NS AS TO MILK TYPE	11.11111
YOGURT, VANILLA, LEMON, COFFEE, WHOLE MILK	11.11111
YOGURT, VANILLA, LEMON, COFFEE, LOWFAT MILK	11.11111
YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK	11.11111
YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK, LOW CAL SWEE	11.11111
YOGURT, CHOCOLATE, NS AS TO TYPE OF MILK	11.11111
YOGURT, CHOCOLATE, WHOLE MILK	11.11111
YOGURT, CHOCOLATE, NONFAT MILK	11.11111
YOGURT, FRUIT VARIETY, NS AS TO MILK TYPE	11.11111
YOGURT, FRUIT VARIETY, WHOLE MILK	11.11111
YOGURT, FRUIT VARIETY, LOWFAT MILK	11.11111
YOGURT, FRUIT VARIETY, NONFAT MILK	11.11111
YOGURT, FRUITED, NONFAT MILK, LOW CAL SWEETENER	11.11111
YOGURT, FRUIT & NUTS, NS AS TO TYPE OF MILK	11.11111
YOGURT, FRUIT & NUTS, LOWFAT MILK	11.11111
YOGURT, FROZEN, NS AS TO FLAVOR, NS TO TYPE OF MILK	6.666667
YOGURT, FROZEN, NOT CHOCOLATE, TYPE OF MILK NS	6.666667
YOGURT, FROZEN, CHOCOLATE, TYPE OF MILK NS	6.666667
YOGURT, FROZEN, NS AS TO FLAVOR, LOWFAT MILK	6.666667
YOGURT, FROZEN, CHOCOLATE, LOWFAT MILK	6.666667
YOGURT, FROZEN, NOT CHOCOLATE, LOWFAT MILK	6.666667
YOGURT, FROZEN, NS AS TO FLAVOR, NONFAT MILK	6.666667
YOGURT, FROZEN, CHOCOLATE, NONFAT MILK	6.666667
YOGURT,FROZEN, NOT CHOCOLATE,W/ SORBET/SORBET-COATED	6.666667
YOGURT, FROZEN, NOT CHOCOLATE, NONFAT MILK	6.666667
YOGURT,FRZ,CHOCOLATE, NONFAT MILK,W/ LOW-CAL SWEET	6.666667
YOGURT,FRZ, NOT CHOC,NONFAT MILK,W/ LOW-CAL SWEET	6.666667
YOGURT, FROZEN, NS AS TO FLAVOR, WHOLE MILK	6.666667
YOGURT, FROZEN, CHOCOLATE, WHOLE MILK	6.666667
YOGURT, FROZEN, NOT CHOCOLATE, WHOLE MILK	6.666667
YOGURT, FROZEN, CHOCOLATE-COATED	6.666667
YOGURT, FROZEN, CAROB-COATED	6.666667
YOGURT, FROZEN, SANDWICH	6.666667
YOGURT, FROZEN, CONE, CHOCOLATE	6.666667
YOGURT, FROZEN, CONE, NOT CHOCOLATE	6.666667
YOGURT, FROZEN, CONE, NOT CHOCOLATE, LOWFAT MILK	6.666667
YOGURT, FROZ, CONE, CHOCOLATE, LOWFAT MILK	6.666667
MILK BEVERAGE, NOT CHOCOLATE, W/ WHOLE MILK	10.41667
MILK SHAKE, NS AS TO FLAVOR OR TYPE	10.41667
MILK SHAKE,HOMEMADE/ FOUNTAIN-TYPE, NS AS TO FLAVOR	10.41667
MILK SHAKE, HOMEMADE OR FOUNTAIN-TYPE, CHOCOLATE	10.41667
MILK SHAKE, HOMEMADE /FOUNTAIN-TYPE, NOT CHOCOLATE	10.41667

Description	Ingredient (mg/g)
MILK SHAKE WITH MALT (INCL MALTED MILK W/ICE CREAM)	10.41667
MILK SHAKE, MADE W/ SKIM MILK, CHOCOLATE	10.41667
MILK SHAKE,MADE W/ SKIM MILK, NOT CHOCOLATE	10.41667
CARRY-OUT MILK SHAKE, NS AS TO FLAVOR	10.41667
CARRY-OUT MILK SHAKE, CHOCOLATE	10.41667
CARRY-OUT MILK SHAKE, NOT CHOCOLATE	10.41667
FRUIT SMOOTHIE DRINK, W/ FRUIT AND DAIRY PRODUCTS	10.41667
FRUIT SMOOTHIE DRINK, NFS	10.41667
DIET BEVERAGE, LIQUID, CANNED	10.41667
DIET BEVERAGE, PWDR,RECONST W/SKIM (INCL CARNATION)	10.41667
COCOA-FLAVORED BEVERAGE POWDER W/ SUGAR, DRY MIX	10.41667
HIGH CALORIE MILK BEVERAGE, POWDER, NOT RECONST	10.41667
MEAL REPLACEMENT, PROTEIN, MILK BASED,FRUIT JUICE MIX	10.41667
ICE CREAM BAR OR STICK, NOT CHOC- OR CAKE-COVERED	6.666667
PUDDING, NFS	6.666667
PUDDING, BREAD (INCLUDE W/ RAISINS)	6.666667
HIGH PROTEIN BAR, CANDY-LIKE, SOY & MILK BASE	62.5
BREAD, WHITE	50
BREAD, BATTER	83.33334
BREAD, HIGH PROTEIN	50
BREAD, 100% WHOLE WHEAT	50
COOKIE, BATTER / DOUGH, RAW, NOT CHOCOLATE	83.33334
COOKIE, ALMOND	83.33334
COOKIE, BROWNIE, LOWFAT, W/O ICING	62.5
COOKIE, CHOCOLATE CHIP	83.33334
COOKIE, BAR, W/ CHOCOLATE, NUTS, & GRAHAM CRACKERS	83.33334
COOKIE, OATMEAL	83.33334
COOKIE, OAT BRAN	83.33334
COOKIE, PEANUT	83.33334
COOKIE, DIETETIC, OATMEAL W/ RAISINS	83.33334
COOKIE, DIETETIC, SANDWICH TYPE	83.33334
COOKIE, DIETETIC, SUGAR OR PLAIN	83.33334
PIE, YOGURT, FROZEN	20
PIE, PUDDING, NOT CHOCOLATE	20
BREAKFAST BAR, CEREAL CRUST W/ FRUIT FILLING, LOWFAT	62.5
BREAKFAST BAR, CEREAL CRUST W/ FRUIT FILLING, FAT FREE	62.5
BREAKFAST BAR, DATE, W/ YOGURT COATING	62.5
MEAL REPLACEMENT BAR (INCL SLIM FAST BAR)	62.5
GRANOLA BAR W/ OATS, SUGAR, RAISINS, COCONUT	62.5
GRANOLA BAR, OATS, FRUIT, NUTS, LOWFAT	62.5
GRANOLA BAR, NONFAT	62.5
GRANOLA BAR W/ PEANUTS, OATS, SUGAR, WHEAT GERM	62.5
GRANOLA BAR, CHOCOLATE-COATED	62.5
GRANOLA BAR, W/ COCONUT, CHOCOLATE-COATED	62.5
GRANOLA BAR W/ NUTS, CHOCOLATE-COATED	62.5
GRANOLA BAR, COATED W/ NONCHOCOLATE COATING	62.5
GRANOLA BAR, HIGH FIBER, YOGURT COATING, NOT CHOC	62.5
GRANOLA BARS, W/ RICE CEREAL	62.5
POWERBAR (FORTIFIED HIGH ENERGY BAR)	62.5
MULTIGRAIN MIX, BREAD STICKS, SESAME NUGGETS, PRETZ	166.6667
NOODLES, COOKED, NS AS TO ADDED FAT	17.85714

Description	Ingredient (mg/g)
NOODLES, COOKED, NO FAT ADDED	17.85714
NOODLES, COOKED, FAT ADDED	17.85714
NOODLES, COOKED,WHOLE WHEAT,NS AS TO FAT ADDED	17.85714
NOODLES, WHOLE WHEAT, COOKED, NO FAT ADDED	17.85714
NOODLES, COOKED, SPINACH, NS AS TO FAT	17.85714
NOODLES, SPINACH, COOKED, NO FAT ADDED	17.85714
NOODLES, COOKED, SPINACH, FAT ADDED	17.85714
NOODLES, CHOW MEIN	100
LONG RICE NOODLES(FROM MUNG BEANS),CKD,NS FAT ADDed	17.85714
LONG RICE NOODLES, COOKED, NO FAT ADDED	17.85714
LONG RICE NOODLES, COOKED, FAT ADDED	17.85714
CHOW FUN RICE NOODLES,COOKED,NS AS TO FAT ADDED	17.85714
CHOW FUN RICE NOODLES, COOKED, NO FAT ADDED	17.85714
CHOW FUN RICE NOODLES, COOKED, FAT ADDED	17.85714
RICE DESSERT BAR,FRZ,NOT CHOC,NONDAIRY,CAROB,COVER	62.5
RICE DESSERT BAR,FRZ,CHOC,NONDAIRY,CHOC COVERED	62.5
WHOLE WHEAT CEREAL, W/ BARLEY, COOKED, NO FAT ADDED	10
WHOLE WHEAT CEREAL, WHEAT & BARLEY, FAT ADDED	83.33334
WHOLE WHEAT CEREAL, WHEAT & BARLEY, ADDED FAT NS	83.33334
COOKIE-CRISP CEREAL (INCLUDE ALL FLAVORS)	45.45454
LASAGNA W/ MEAT, WHOLE WHEAT NOODLES	10
LASAGNA, MEATLESS, WHOLE WHEAT NOODLES	10
LASAGNA W/ MEAT, SPINACH NOODLES	10
LASAGNA, MEATLESS, SPINACH NOODLES	10
MACARONI OR NOODLES W/ CHEESE	10
ORANGE SECTIONS, CANNED, JUICE PACK	17.85714
ORANGES, MANDARIN, CANNED OR FROZEN, JUICE PACK	17.85714
ACEROLA JUICE	10.41667
GRAPEFRUIT JUICE, NFS	10.41667
GRAPEFRUIT JUICE, FRESHLY SQUEEZED	10.41667
GRAPEFRUIT JUICE, UNSWEETENED, NS AS TO FORM	10.41667
GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, UNSWEET	10.41667
GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, W/ SUGAR	10.41667
GRAPEFRUIT JUICE, FROZEN, UNSWEETENED (RECONST)	10.41667
LEMON JUICE, NS AS TO FORM	500
LEMON JUICE, FRESH	500
LEMON JUICE, CANNED OR BOTTLED	500
LEMON JUICE, FROZEN	500
LIME JUICE, NS AS TO FORM	500
LIME JUICE, FRESH	500
LIME JUICE, CANNED OR BOTTLED	500
LIME JUICE, FROZEN	500
ORANGE JUICE, NFS	10.41667
ORANGE JUICE, FRESHLY SQUEEZED	10.41667
ORANGE JUICE, CANNED/BOTTLED/CARTON, UNSWEETENED	10.41667
ORANGE JUICE, CANNED/BOTTLED/CARTON, W/ SUGAR	10.41667
ORANGE JUICE, W/ CALCIUM, CAN/BOTTLE/CARTON, UNSWEETENED	10.41667
ORANGE JUICE, FROZEN, UNSWEETENED, RECONST W/ WATER	29.41176
ORANGE JUICE, FROZEN, W/ SUGAR, RECONST W/ WATER	29.41176
ORANGE JUICE, FROZEN, UNSWEETENED, NOT RECONSTITUTD	29.41176
ORANGE JUICE, FROZEN, W/ SUGAR, NOT RECONSTITUTED	29.41176

Description	Ingredient (mg/g)
ORANGE JUICE,FROZ, W/,CALCIUM ADDED,RECON W/WATER	29.41176
TANGERINE JUICE, NFS	10.41667
TANGERINE JUICE, CANNED, UNSWEETENED	10.41667
TANGERINE JUICE, FROZEN, UNSWEET, RECONST W/ WATER	10.41667
GRAPE-TANGERINE-LEMON JUICE	10.41667
GRAPEFRUIT & ORANGE JUICE, NFS	10.41667
GRAPEFRUIT & ORANGE JUICE, FRESH	10.41667
GRAPEFRUIT & ORANGE JUICE, CANNED, UNSWEETENED	10.41667
GRAPEFRUIT & ORANGE JUICE, CANNED, W/ SUGAR	10.41667
GRAPEFRUIT & ORANGE JUICE, FROZEN, (RECONSTITUTED)	29.41176
ORANGE & BANANA JUICE	10.41667
ORANGE-WHITE GRAPE-PEACH JUICE	10.41667
APRICOT-ORANGE JUICE	10.41667
PINEAPPLE-GRAPEFRUIT JUICE, NFS	10.41667
PINEAPPLE-GRAPEFRUIT JUICE, CANNED, NS SWEETENED	10.41667
PINEAPPLE-GRAPEFRUIT JUICE, CANNED, UNSWEETENED	10.41667
PINEAPPLE-GRAPEFRUIT JUICE, CANNED, W/ SUGAR	10.41667
PINEAPPLE-GRAPEFRUIT JUICE, FROZEN, RECONST W/WATER	29.41176
PINEAPPLE-ORANGE JUICE, NFS	10.41667
PINEAPPLE-ORANGE JUICE, CANNED, NS AS TO SWEETENER	10.41667
PINEAPPLE-ORANGE JUICE, CANNED, UNSWEETENED	10.41667
PINEAPPLE-ORANGE JUICE, CANNED, W/ SUGAR	10.41667
PINEAPPLE-ORANGE JUICE, FROZEN, RECONST W/ WATER	29.41176
STRAWBERRY-BANANA-ORANGE JUICE	10.41667
APRICOT, COOKED OR CANNED, JUICE PACK	17.85714
CHERRIES, SWEET, COOKED OR CANNED, JUICE PACK	17.85714
PEACH, COOKED OR CANNED, JUICE PACK	17.85714
PEAR, COOKED OR CANNED, JUICE PACK	17.85714
PINEAPPLE, COOKED OR CANNED, JUICE PACK	17.85714
PLUM, COOKED OR CANNED, JUICE PACK	17.85714
FRUIT JUICE BAR, FROZEN, FLAVOR OTHER THAN ORANGE	29.41176
FRUIT JUICE BAR, FROZ, LOW CAL SWEETNER, NOT ORANGE	29.41176
FRUIT JUICE BAR W/ CREAM, FROZEN	29.41176
FRUIT JUICE, NFS (INCLUDE MIXED FRUIT JUICES)	10.41667
FRUIT JUICE BLEND, 100% JUICE, W/ VITAMIN C	10.41667
AMBROSIA JUICE (INCL KNUDSEN'S)	10.41667
APPLE JUICE	10.41667
APPLE JUICE, W/ ADDED VITAMIN C	10.41667
APPLE JUICE WITH ADDED VITAMIN C AND CALCIUM	10.41667
APPLE-CHERRY JUICE	10.41667
APPLE-PEAR JUICE	10.41667
APPLE-RASPBERRY JUICE	10.41667
APPLE-GRAPE JUICE	10.41667
APPLE-GRAPE-RASPBERRY JUICE	10.41667
BLACKBERRY JUICE (INCL BOYSENBERRY JUICE)	10.41667
CRANBERRY JUICE, UNSWEETENED	10.41667
CRANBERRY-WHITE GRAPE JUICE MIXTURE, UNSWEETENED	10.41667
GRAPE JUICE, NFS	10.41667
GRAPE JUICE, UNSWEETENED	10.41667
GRAPE JUICE, W/ SUGAR	10.41667
GRAPE JUICE, LOW CALORIE SWEETENER	10.41667

Description	Ingredient (mg/g)
GRAPE JUICE, NS AS TO SWEETENED, W/ ADDED VITAMIN C	10.41667
GRAPE JUICE, UNSWEETENED, W/ ADDED VITAMIN C	10.41667
GRAPE JUICE, W/ SUGAR, W/ ADDED VITAMIN C	10.41667
PAPAYA JUICE	10.41667
PASSION FRUIT JUICE	10.41667
PEACH JUICE, W/ SUGAR	10.41667
PINEAPPLE JUICE, NS AS TO SWEETENED	10.41667
PINEAPPLE JUICE, UNSWEETENED	10.41667
PINEAPPLE JUICE, W/ SUGAR	10.41667
PINEAPPLE JUICE, UNSWEETENED, W/ VIT C	10.41667
PINEAPPLE-APPLE-GUAVA JUICE, W/ ADDED VITAMIN C	10.41667
PINEAPPLE JUICE-NON-CITRUS JUICE BLEND, UNSWEETENED	10.41667
PRUNE JUICE, NS AS TO ADDED SWEETENER	10.41667
PRUNE JUICE, UNSWEETENED	10.41667
PRUNE JUICE, W/ SUGAR	10.41667
STRAWBERRY JUICE	10.41667
WATERMELON JUICE	10.41667
CARROT JUICE	10.41667
TOMATO JUICE	10.41667
TOMATO JUICE, LOW SODIUM	10.41667
TOMATO JUICE COCKTAIL	10.41667
TOMATO & VEGETABLE JUICE, MOSTLY TOMATO (INCL V-8)	10.41667
TOMATO & VEGETAGLE JUICE, MOSTLY TOMATO, LOW SODIUM	10.41667
CELERY JUICE	10.41667
ALOE VERA JUICE	10.41667
FRUIT CANDY BAR	83.33334
PEANUT CANDY BAR	62.5
PEANUT BAR, CHOCOLATE COVERED CANDY	62.5
COFFEE, DECAFFEINATED, W/ CEREAL (INCLUDE W/BARLEY)	10.41667
CEREAL, BEVERAGE (INCLUDE PERO, BREAK AWAY)	10.41667
CEREAL BEVERAGE, W/BEET ROOTS, FROM POWDERED INSTANT	10.41667
CEREAL BEVERAGE, W/BEET ROOTS, POWDERED INSTANT, DRY	10.41667
MATE, SWEETENED BEVERAGE FROM DRIED GREEN LEAVES	10.41667
CARBONATED WATER, UNSWEETENED (INCL CLUB SODA)	10.41667
CREAM SODA	10.41667
CREAM SODA, SUGAR-FREE	10.41667
CHOCOLATE-FLAVORED SODA	10.41667
CHOCOLATE-FLAVORED SODA, SUGAR-FREE	10.41667
CARBONATED JUICE DRINK, NS AS TO TYPE OF JUICE	10.41667
CARBONATED CITRUS JUICE DRINK	10.41667
CARBONATED NONCITRUS JUICE DRINK	10.41667
APPLE JUICE DRINK	10.41667
APPLE-CRANBERRY-GRAPE JUICE DRINK	10.41667
APPLE-ORANGE-PINEAPPLE JUICE DRINK	10.41667
APRICOT-PINEAPPLE JUICE DRINK	10.41667
FRUIT JUICE DRINK, NFS	10.41667
FRUIT PUNCH, MADE W/ FRUIT JUICE & SODA	10.41667
FRUIT PUNCH, MADE W/ SODA, FRUIT JUICE & SHERBET	10.41667
GRAPE JUICE DRINK	10.41667
GRAPEFRUIT JUICE DRINK	10.41667
GUAVA JUICE DRINK	10.41667

Description	Ingredient (mg/g)
ORANGE-MANGO JUICE DRINK	10.41667
ORANGE-APRICOT JUICE DRINK	10.41667
CITRUS FRUIT JUICE DRINK (INCL 5-ALIVE)	10.41667
ORANGE-CRANBERRY JUICE DRINK	10.41667
ORANGE-PEACH JUICE DRINK	10.41667
ORANGE-GRAPE-BANANA JUICE DRINK	10.41667
PAPAYA JUICE DRINK	10.41667
PINEAPPLE-GRAPEFRUIT JUICE DRINK	10.41667
PINEAPPLE-ORANGE JUICE DRINK	10.41667
ORANGE-RASPBERRY JUICE DRINK	10.41667
PINA COLADA, NONALCOHOLIC	10.41667
WHISKEY SOUR, NONALCOHOLIC (INCL LEMIX)	10.41667
CRANBERRY JUICE DRINK W/VIT C ADDED (INCL COCKTAIL)	10.41667
CRANBERRY-APPLE JUICE DRINK W/ VITAMIN C ADDED	10.41667
GRAPEFRUIT JUICE DRINK W/ VITAMIN C ADDED	10.41667
GUAVA JUICE DRINK W/ VIT C ADDED	10.41667
VEGETABLE & FRUIT JUICE DRINK, W/ VIT C	10.41667
PINEAPPLE-GRAPEFRUIT JUICE DRINK W/ VIT C ADDED	10.41667
PINEAPPLE-ORANGE JUICE DRINK W/ VITAMIN C ADDED	10.41667
PINEAPPLE-ORANGE-GRAPEFRUIT JUICE DRINK W/VITAMIN C	10.41667
APPLE-WHITE GRAPE JUICE DRINK, LOW CAL, W/VIT C ADDED	10.41667
CRANBERRY JUICE COCKTAIL, LO CAL, W/ VIT C ADDED	10.41667
CRANBERRY-APPLE JUICE DRINK, LO CAL, VIT C ADDED	10.41667
GRAPEFRUIT JUICE DRINK, LOW CALORIE, W/ VITAMIN C	10.41667
JUICE DRINK, LOW CALORIE	10.41667
ORANGE-CRANBERRY JUICE DRINK, LOW CAL, W/ VIT C ADDED	10.41667
FRUIT-FLAVORED THIRST QUENCHER BEVERAGE, LOW CAL	10.41667
FRUIT-FLAVORED THIRST QUENCHER BEVERAGE	10.41667
CITRUS JUICE DRINK, CALCUIM FORTIFIED	10.41667
HORCHATA, P.R. (BEVERAGE)	10.41667
COCONUT BEVERAGE, P.R.	10.41667
OATMEAL BEVERAGE, P.R.	10.41667
OATMEAL BEVERAGE W/ MILK	10.41667
RICE BEVERAGE, MEXICAN (HORCHATA)	10.41667
GRANOLA BAR, HIGH FIBER, YOGURT COATING, NOT CHOC	62.5
FRUIT PUNCH, MADE W/ SODA, FRUIT JUICE & SHERBET	10.41667

Foods in this Appendix are foods that are representative of the foods to which PGX will be added. Regardless of the name brands, the specific list does not restrict addition of PGX to other brands represented under that description. These foods are only foods found in the NHANES dietary database, and represent examples of the types of foods to which PGX will be added.

APPENDIX B

Table A. Individual lot analysis data for PGX

Analysis	Lot # 1282 060320	Lot # 1285 060330	Lot # 1318 060420	Lot # 1752 070105	Lot # 2029 070523
Carbohydrates (%)	82	82.9	83	84.7	85.4
Total Dietary Fibre (%)	82.4	91.6	82.3	83.5	83.9
Ash (total) (%)	5.7	5	6.2	5.9	5.8
Protein (%)	2	1.8	2.1	2.2	2.1
Fat (%)	<0.1	0.1	0.1	<0.1	<0.1
Loss on drying (%)	9.08	9.03	6.47	4.2	4.54
Arsenic	<1.5 ppm	<1.5 ppm	<1.5	<1.5	<1.5 ppm
Lead	<0.5 ppm	0.51	0.68	<0.5	0.92 ppm
Sodium	2.44	2.48	2.94	2.7	2.34
Potassium	0.52	0.48	0.8	0.8	0.41
Microbiological (cfu/g)					
Standard Plate Count	20	80	400	80	700
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Salmonella spp	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative	Negative	Negative
Yeast and Mold	<10, <10	<10,<10	<10,<10	<10, <10	<10,<10
Date of manufacture	Jun-06	Mar-06	Apr-06	Jan-07	May-07

cfu = colony forming units; ppm = parts *per* million

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AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

October 15, 2009

FINAL

Panel Members

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AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

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AMENDMENT TO THE DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF POLYGLYCOPLEX® (PGX®) AS A FOOD INGREDIENT

1. EXECUTIVE SUMMARY

InovoBiologic Inc. (hereinafter referred to as Inovo) convened an independent Panel of Experts (Expert Panel) [1] to critically evaluate the available information on PolyGlycopleX® (PGX®). The information evaluated included the results of a search of the scientific literature through September 2008 (conducted by the Burdock Group) and other information provided by Inovo. Following its critical evaluation of available information, the Expert Panel concluded in January 2009 that, according to the provision of the Federal Food Drug and Cosmetic Act (FD&C Act) and the guidance provided by 21CFR§170.30 and 21CFR§170.35, the intended use of PGX® as an ingredient to add fiber to the diet, is Generally Recognized As Safe (GRAS), based on scientific procedures.[2]

Inovo recently requested the Burdock Group to amend the original PGX® GRAS determination to include a new food category, medical foods, for use by a select population that is under physician's care and supervision. Burdock Group analyzed the literature and the criteria of medical food use and determined that consumption of PGX® as an ingredient added to medical food would not exceed the safe consumption of PGX® as described in the original GRAS dossier. The Burdock Group then presented the information to a panel of experts. The GRAS Expert Panel evaluated this new information and concluded that the safety data provided in the original GRAS dossier continues to support a conclusion of safety. And, further, a critical review of the literature published since the original PGX® GRAS determination did not reveal any new safety information that would affect the original determination of GRAS status. The GRAS Expert Panel concluded that this additional use of PGX®, as an ingredient for use in medical food, is Generally Recognized As Safe (GRAS) based on scientific procedures.

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[1] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.

[2] "Dossier in support of the Generally Recognized As Safe (GRAS) status of PolyGlycopleX® as a food ingredient", finalized as a GRAS determination on January 8, 2009.

2. INTRODUCTION

PGX® is manufactured from three water soluble polysaccharides: konjac powder,[3] sodium alginate, and xanthan gum. The polysaccharides used in its production are complementary to each other and act synergistically to form strong interactions that lead to a level of viscosity that is 3 – 5 times higher than any other known polysaccharide (InovoBiologic, 2007). Viscous soluble fibers thicken when mixed with fluids and have been associated with lowered cholesterol and blood glucose concentrations, slower transit time through the gastrointestinal tract, and prolonged gastric emptying (Dikeman and Fahey, 2006). The Institute of Medicine (IOM, 2005) recommended an adequate intake of fiber for women of 25 g fiber/day, and an adequate intake for men of 38 g fiber/day, as part of a healthy diet. However, estimates indicate that dietary fiber intakes range from 12.1 – 13.8 g/day for women and 16.5 – 17.9 g/day for men (IOM, 2005). The consumption of fiber in the diet can exert a normalizing effect on the GI[4] functions. As an example, fiber's adsorptive effect can add bulk to the consistency of stool, decreasing the formation of diarrhea from excessive GI motor function, while fiber's absorptive capacity may alter glucose and cholesterol metabolism. If the GI transit time is too slow, constipation may result; but fiber enhances peristaltic activity, decreasing transit time in the GI tract (Friedman *et al.*, 1990). PGX® has previously been determined GRAS when added to a variety of food products to provide added fiber to the diet. This amendment is to determine the safe use of PGX® as an ingredient in medical foods to provide those consumers directly under a physician's care an additional source of fiber.

The term "medical food" was initially defined in the Orphan Drug Act Amendments of 1988 [21 USC 360ee (b)(3)].[5] This definition was incorporated by reference into the Nutrition Labeling and Education Act (P.L. 101-535) in November 1990, and it was incorporated into the agency's final rule on Mandatory Nutritional Labeling in January 1993. The definition for a medical food is:

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[3] Konjac powder is also known as konjac flour.

[4] GI = gastrointestinal

[5] http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/MedicalFoods/ucm054048.htm (site visited August 31, 2009).

> A food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.
> (Food Compliance Program, December 21, 1998. U.S. FDA/CFSAN).

Medical foods may be the sole source of calories and nutrients for a patient. Medical foods are recommended for patients with a variety of medical conditions including, but not limited to, renal disease (*e.g.*, chronic or acute renal failure), hypermetabolic states (*e.g.*, severe burns, trauma or infections), and malabsorption (*e.g.*, inflammatory bowel disease, irritable bowel syndrome, diverticulosis and short bowel syndrome). On occasion, certain medical foods may be consumed as a caloric and nutritive supplement (*i.e.*, may be taken to supplement the typical diet), as a result of the patient's specific disease or condition. As an example, individuals with dysphagia (difficulty in swallowing) are typically provided fluids with added thickeners to prevent aspiration (Sharpe *et al.*, 2007).

2.1. Description

PGX® is an off-white granular powder composed of a proprietary complex of the food-grade water-soluble polysaccharides konjac powder, sodium alginate, and xanthan gum. These polysaccharides are processed in a conditioned chamber to produce an interlocking matrix of the three components (*i.e.*, the complex). The polysaccharides used in the production of PGX® act synergistically to form strong interactions that lead to a level of viscosity that is 3 – 5 times higher than the individual components or any other currently known individual polysaccharide. These three polysaccharides interact to form a novel ingredient rather than a mixture of three separate components by forming junction zones and networks between the raw ingredients which prevent the individual components from exhibiting the properties that they would show in their pure state (Lawson *et al.*, 2009). The specifications and manufacturing process outlined in the original GRAS determination have not changed.[2]

2.2. Current uses

PGX® is currently used as a dietary supplement and as an added fiber ingredient in the food categories shown in Table 1 *per* the original GRAS dossier.

Table 1. Food categories for the addition of PGX® *per* the original GRAS dossier[2]

Food Category
Yogurts
Milk shakes and fruit smoothie-type drinks
Frozen yogurt, ice cream bar, and puddings
White and whole wheat breads
Cookies
Breakfast bars
Granola-type bars
Noodles
Whole wheat cereals
Lasagna and macaroni/cheese
Fruit juices and fruit juice bars
Cereal beverage

2.3. Intended use or uses

PGX® is intended to be used as a source of dietary fiber in conventional foods and medical foods. PGX® in medical foods will be used by a select population that is under physician's care and supervision, for daily intake not to exceed 13,070 mg, the same level determined GRAS in the original GRAS determination. The label on the product must clearly state that the product is intended for use in the management of a specific medical disorder or condition. The addition of PGX® cannot result in a food becoming a medical food, as this substance will be added to foods already determined to be medical foods.

3. ESTIMATED DAILY INTAKE

An understanding of the consumption of the intended new use of a food ingredient, as an index of consumer exposure at ingredient use level(s) in various food group(s), is key to any food safety risk assessment process. An Expert Panel previously determined that the intended uses of PGX® were GRAS for use in the foods specified and at the concentrations provided in Table 2, resulting in an estimated mean intake of 5019 mg/day PGX® and a 90th percentile consumption at 10,070 mg/day (167.8 mg/kg/day for a 60 kg person). PGX® is also available to the public as a dietary supplement, with the suggested serving of 3000 mg/day.

Table 2. Food groups selected for PGX® supplementation*

Food Category	Intended use level (ppm)
Yogurts	11,110
Milk shakes and fruit smoothie-type drinks	10,420
Frozen yogurt, ice cream bar, and puddings	6,670
White and whole wheat breads	50,000
Cookies	83,330
Breakfast bars	62,500
Granola-type bars	62,500
Noodles	17,860
Whole wheat cereals	83,330
Lasagna and macaroni/cheese	10,000
Fruit juices and fruit juice bars	10,420
Cereal beverage	10,420

*The food categories correspond to those listed in 21 CFR 170.3(n); ppm=parts *per* million

The intended new use of PGX® is as a dietary fiber source in medical foods for use by a select population that is under physician's care and supervision, for an aggregate daily intake not to exceed 13,070 mg, as monitored by the health care provider. Precise exposure estimates for this new use of PGX® in medical foods is not possible since medical foods are intended only for patients receiving active and ongoing medical treatment and supervision. Although the patient will receive instructions on the use of the medical food, it is the physician who will determine the sources of fiber in the diet of the patient.

The addition of PGX® to products previously designated as medical foods intended as the sole dietary source may be formulated to provide a total daily consumption of 13,070 mg PGX®. Medical foods that are to be consumed as part of a complete, balanced diet are typically formulated to replace one or more meals throughout the day, thereby decreasing the amount of fiber consumed from conventional foods. Therefore, medical food products consumed as part of a balanced diet under the direct supervision of a physician may contain PGX® such that the aggregate amount of PGX® consumed in the daily diet would be limited to 10,070 mg/day (*e.g.*, the attending physician will determine the total intake of PGX®, which may be less than 13,070 mg/day). Patients receiving such a medical food as a sole source of nutrition would not exceed IOM's recommended daily intake of dietary fiber because of the absence of other fiber sources. The Expert Panel determined that supplemental uses to the typical diet (if the medical food is not

the sole source of the diet) to also be consistent with the safe use of PGX® for the following reasons:

- The original GRAS determination evaluated the total consumption of PGX® from the addition to foods and as a dietary supplement, with the total mean and 90th percentile consumption when added to foods at 5019 and 10,070 mg/day, respectively (Table 3). Dietary supplement use is suggested at 3000 mg/day. The total consumption of PGX® from foods containing PGX® and as a dietary supplement was stated in the original GRAS at 13,070 mg/day. The Expert Panel reasoned that if a patient is receiving medical food to supplement his/her intake of a substance, such as fiber, or to replace a complete meal from the overall diet, it is unlikely that the patient will be consuming enough conventional food to achieve the mean or 90th percentile intakes of PGX®, therefore decreasing the amount of PGX® consumed from foods while consuming a similar amount of PGX® from the medical food product.

Table 3. Predicted intake of PGX® following supplementation of selected foods at the indicated levels (Table 2) and dietary supplements[2]

PGX® intake from:	*Per* User (mg/day)	
	Mean	90th Percentile
Possible maximum consumption as an added ingredient to food (conventional or medical)*	5019	10,070
Possible consumption as a dietary supplement**	3000	3000
Total consumption from food and dietary supplements	8019	13,070

* Medical foods containing PGX® would either be consumed as a sole source of the diet, or would replace the amount of PGX® consumed from conventional foods; **Consumption according to label directions

- The totals above assume consumption of PGX® as a dietary supplement at 3000 mg/day. Because medical foods are administered under supervision of a physician, the Expert Panel reasoned that patients who receive medical food to supplement their caloric and nutritive intake would be unlikely to consume additional dietary supplements containing fiber (PGX® in a medical food and in a dietary supplement would provide approximately 16 g PGX®), at least to the extent of the exaggerated estimates identified above. The documented effects of over-consumption of fiber include the potential for reduced bioavailability of minerals such as iron, calcium and

zinc, especially when phytate is present (appreciable levels of phytate are not present in PGX®), and may produce effects on the GI tract related to fiber intolerance, such as flatulence, diarrhea, constipation, and gastrointestinal distress. It has been reported (Dutta and Hlasko, 1985) that consumption of dietary fiber at levels of 75 – 80 g/day for 6-7 days was associated with a sensation of excessive abdominal fullness and increased flatulence in individuals with pancreatic disease,[6] and a reported case of intestinal obstruction occurred in a women on antidepressants that consumed 160 – 200 g/day of unprocessed bran for six months (Kang and Doe, 1979). Consumption of fiber (*i.e.*, wheat bran) at levels up to 40 g/day in a clinical trial with 60 subjects did not result in significant increases in gastrointestinal distress (McRorie *et al.*, 2000), compared to controls, while consumption of resistant starch at 32 g/day for one week (Heignen *et al.*, 1998) resulted in increased flatulence (IOM, 2005). The inclusion of PGX® into a medical food, either as a sole source of the diet, or as part of a well-balanced diet, is not expected to cause significant effects related to tolerance.

It is highly unlikely that an individual would be consuming foods enriched with PGX®, taking PGX® as a dietary supplement, and be consuming medical foods containing PGX®. However, in the unlikely event that this would occur, the 90th percentile consumption of PGX® would be 16,070 mg/day. High levels of fiber consumption (approximately 20 – 40 g additional fiber/day) are not related to any serious chronic adverse effects.

4. PRECLINICAL AND CLINICAL STUDIES

The consumption of fiber, in general, may increase mild, unpleasant gastrointestinal symptoms, such as flatulence, feelings of bloatedness, cramping, and diarrhea, but serious chronic adverse effects have not been reported. IOM (2005) provided a general overview of studies on the effects of the consumption of isolated and synthetic fibers, including studies in which polydextrose was administered at 12 g/day (n = 66 men and 54 women) for 28 days with no reports of abdominal cramping or diarrhea (Jie *et al.*, 2000), and no complaints of abdominal

[6] Twelve patients with exocrine pancreatic insufficiency secondary to chronic alcoholic pancreatitis. Patients with pancreatic insufficiency may be placed on a high fiber diet, as dietary fiber has been demonstrated to have a positive influence on the control of diabetes mellitus.

distress with the consumption of 30 g/day polydextrose ($n = 7$ healthy male subjects) for up to 21 days (Achour *et al.*, 1994). Adverse effects of psyllium consumption in isolated case reports [*i.e.*, esophageal obstruction in an elderly man with a past history of episodic mild dysphagia, who took a "heaping" teaspoon of psyllium with some water for several years[7] (Noble and Grannis, Jr., 1984), or an elderly woman with small bowel obstruction who consumed 2-3 tablespoons of a psyllium-based laxative three times/day concurrent with a prescription laxative, who was found to have a small-bowel ileus that required surgical intervention (Berman and Schultz, 1980)] were determined to be due to inadequate water consumption with gastrointestinal obstructions. Consumption of resistant starch at up to 32 g/day for three weeks resulted in flatulence and bloated feelings, but no serious adverse effects (Phillips *et al.*, 1995; Heignen *et al.*, 1998). IOM (2005) concluded:

> While occasional adverse gastrointestinal symptoms are observed when consuming some of the isolated or synthetic fibers, serious chronic adverse effects have not been observed. Furthermore, due to the bulky nature of fibers, excess consumption is likely to be self-limiting. Therefore, a UL[8] was not set for these individual fibers.

The following is a summary of additional preclinical and clinical studies published since the initial GRAS determinations conducted in 2008 that evaluate the effect of PGX® or one of the raw ingredients used to manufacture PGX® (*i.e.*, konjac powder, sodium alginate, xanthan gum).

4.1. Preclinical studies

The actual digestibility of certain carbohydrates identified as "low-digestible" has not been clearly defined in the literature. Knapp *et al.* (2008) conducted a series of studies to determine the digestibility and gastrointestinal tolerance to several low-digestible carbohydrates, including xanthan gum. In an *in vitro* model of simulated gastric digestion[9], xanthan gum produced a low concentration of released monosaccharides, indicating that xanthan gum has a

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[7] The man was found to have an esophageal hiatus hernia and Shatzki ring, a narrow ring of tissue located just above the junction of the esophagus and stomach.

[8] UL = upper limit

[9] Approximately 200 mg of xanthan gum was incubated with pepsin/hydrochloric acid, amylogucosidase, and a-amylase to simulate gastric and small intestinal digestion.

low level of digestibility. When compared to polydextrose, resistant starch and pullulan, xanthan gum was the most resistant to digestion. In an *in vivo* study in dogs analyzing the gastrointestinal tolerance of different low-digestible carbohydrates, female dogs with hound bloodlines (n = 9, initial body weight at 25.1 kg) were fed xanthan gum for a total of ten days. Xanthan gum was incorporated into a 250 g diet (36 g of the daily diet was xanthan gum) which was provided initially; however, this amount was inadequate to maintain weight and therefore the food intake was first increased to 300 g/day (43.5 g xanthan gum), then to 350 g/day (50 g xanthan gum, approximately 2000 mg/kg bodyweight/day). The dogs were randomly assigned into a balanced incomplete block design with two blocks of nine dogs each (n = 18), and within each block the dogs were allotted to one of three diets: control, 100% of the acceptable intake (AI) and 200% of the AI of fiber. The diet was formulated so that 250 g/day provided 100 or 200% of the AI of dietary fiber; the increase in food intake resulted in dogs consuming more than the recommended AI (approximately 140 or 240% of the AI). Each block was conducted over a ten-day period, with Days 1-7 were assigned to diet acclimation, followed by an evaluation of tolerance characteristics on Days 8-10 (*i.e.*, the dogs consumed the diet for a 10-day period, but the tolerance evaluation period was only conducted during the final three-day period). The fiber intakes were formulated to provide approximately 18 and 36 g/day quantities of fiber to equate to 100 and 200% AI amounts of dietary fiber for humans. Ingestion of xanthan gum did not affect food intake, body weight gains, behavior or appearance. Fecal scores increased[10] with increasing levels of xanthan gum consumption, but xanthan gum did not result in diarrhea (but some dogs tolerated loose stools).

Razavi *et al.* (2008) evaluated the therapeutic efficacy of a low viscosity sodium alginate (LVA) solution as the sole source of drinking water (0.5% w/v,[11] approximately equivalent to 0.65-0.77 mg/kg bw/day LVA) in female Sprague-Dawley rats for up to six weeks after colitis (*i.e.,* inflammatory bowel disease, IBD) induction with trinitrobenzene sulfonic acid (TNBS). TNBS administration resulted in acute (*i.e.*, Week 1) and subchronic (*i.e.*, Week 6) IBD in control (non-LVA-treated) rats. Rats administered LVA after TNBS treatment had significantly

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[10] Scores based on the following scale: 1 = dry, hard pellets; 2 = dry, well-formed stool; 3 = soft, moist, formed stool; 4 = unformed stool; and 5 = watery liquid that can be poured.

[11] w/v = weight/volume

reduced colonic damage scores, compared with TNBS-treated rats administered water, at 1, 2, 3, and six weeks post induction of colitis. The authors concluded that "LVA therapy palliated the progression of colonic inflammatory lesions in an experimental model of IBD" (Razavi *et al.*, 2008).

4.2. Genotoxicity and cytotoxicity

The potential genotoxicity of PGX® has been studied in the bacterial reverse mutation assay (Ames test) and in the mammalian erythrocyte micronucleus test (Marone *et al.*, 2009). In the bacterial reverse mutation assay, the highest possible concentration of PGX® that could be prepared (due to the insolubility of PGX® in organic solvents and gelling capacity in aqueous solutions) was a suspension of 1 mg/ml in distilled water. Therefore, the maximum concentration of PGX® was 100 µg/plate. PGX® was evaluated at 0, 0.0316, 0.100, 0.316, 1.0, 3.16, 10.0, 31.6, and 100 µg/plate, both with and without metabolic activation (rat liver microsomal S9 fraction). PGX® was evaluated in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA, consistent with OECD[12] guideline No. 471. PGX® was not cytotoxic at any concentration, and did not induce a significant increase in gene mutations in the bacterial strains utilized in this study.

For the mammalian erythrocyte micronucleus experiment, PGX® was extracted *via* agitation in cottonseed oil for 72 hours with a mass/volume ratio of 0.2 g/ml. The supernatant extract was isolated without centrifugation or filtration and diluted with cottonseed oil (the 100% extract was considered the maximum tolerated dose (1xMTD), with dilutions made at 50% (0.5xMTD) and 20% (0.2xMTD) of the MTD). The extract was administered intraperitoneally (*i.p.*) once at 10 ml/kg body weight to 7- 12-week-old NMRI mice (five males and five females *per* dose group). Peripheral blood was obtained from the mice 44 hours following treatment with the test sample and the positive control group, and 68 hours for the 1xMTD dose group, and evaluated for an increase in the formation of micronuclei in polychromatic erythrocytes (PCE) and the ratio between immature and mature erythrocytes (relative PCE). Administration of the PGX® extract at any dose did not increase the percentage of PCE or relative PCE, compared to

[12] Organisation for Economic Co-operation and Development

control values. The authors stated that administration of the PGX® extract *i.p.* did induce transient toxic effects in the 1xMTD dose group. These transient effects included a reduction of spontaneous activity, increase in the prone position, rough fur, palpebral closure, and constricted abdomen, and were observed at four hours post administration, but these effects were absent by 68 hours post dose. The 0.5xMTD and 0.2xMTD dose groups also showed signs of transient toxicity, but the authors stated that these toxic symptoms were "moderately or weakly developed, only mild instances of rough fur being manifested in both males and females after four hours." Overall, the authors concluded that "these genotoxicity studies show PGX to be nonmutagenic in both the Ames bacterial reverse mutation assay and the mammalian erythrocyte micronucleus test" (Marone *et al.*, 2009).

Yeh *et al.* (2007) compared the effects of konjac glucomannan and a fiber-free diet on the toxicity of fecal water to colon cells. Konjac glucomannan (5% w/w[13]) was administered to male Balb/C mice for three weeks (approximately equivalent to 7500 mg/kg body weight/day) and the feces obtained during Days 18 – 21 were used to prepare a fecal water solution,[14] which was added to Caco-2[15] cells and incubated for three hours. The cells were then harvested and analyzed *via* the comet assay for DNA damage. The fiber-free fecal water significantly decreased cell viability (compared to control buffer) and caused DNA damage. The fecal water from the konjac-fed mice significantly suppressed the decreased cell viability and DNA damage seen by treatment with the fecal water from the fiber-free diet ($P<0.05$). The authors did not state any direct effects of konjac administration on the Balb/C mice utilized in this study, only that konjac administration significantly increased the proportion of the daily fecal excretion of lactobacilli and bifidobacteria, and decreased the proportion of *Clostridium perfringens* ($P<0.05$). The authors concluded that a diet containing 5% konjac glucomannan "significantly decreased the toxic effects of fecal water obtained from BALB/c mice on Caco-2 cells."

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[13] weight/weight

[14] The lyophilized fecal composites were rehydrated to three-fold their original fecal weight, then centrifuged at 36,000x*g* for two hours. The resulting liquid was termed fecal water and used immediately after preparation to determine cytotoxicity.

[15] A human colon adenocarcinoma cell line.

4.3. Human studies

Vuksan *et al.* (2009) conducted a double-blind, randomized, controlled crossover trial that evaluated the dietary effects of a single consumption of three different viscous fibers: cellulose, a low viscosity fiber, glucomannan, a medium viscosity fiber, and a novel high-viscous polysaccharide fiber (NVP) in adolescents.[16] The subjects (n = 31, 6 males and 25 females) were of a mean age of 16.1 ± 0.6 years with a body mass index (BMI) of 22.2 ± 3.7 kg/m^2. The fibers were provided in a strawberry-flavored meal-replacement drink provided in two doses, which were shaken vigorously immediately prior to serving. The subjects were allocated a maximum time for consumption. The drinks were identical in taste, texture, appearance, calorie and macronutrient content, the only difference being the viscosity of the fiber. One serving provided 5.1 g dietary fiber. The drink was consumed 90 minutes prior to an *ad libitum* pizza meal. A physical comfort questionnaire was completed that included gastrointestinal symptoms of bloating, belching, diarrhea, flatulence, and nausea. All fiber preloads decreased the average appetite scores at 15 minutes (P<0.001), but there was no difference between the three different fiber substances. There were no differences between substances in ratings of bloating, belching, flatulence, diarrhea and nausea during the testing periods, with the median ratings for physical comfort at 0 on a visual analog scale, with the ranking of 0 equal to a low severity of the symptom, and 100 indicating a "high" severity of the symptom. This study indicates that the consumption of PGX® at 5.1 g/serving (approximately 85 mg/kg bw[17]/day for a 60 kg person) did not result in a significant increase in adverse tolerance effects (Vuksan *et al.*, 2009).

Konjac has recently been evaluated for its effects on bowel habits and colonic ecology of seven constipated female subjects (45.9 ± 2.7 years of age; body weight at 55.0 ± 1.0 kg, with 15.6 ± 7.6 year history of constipation) (Chen *et al.*, 2008). The subjects were identified as constipated if they passed a bowel movement less than once a day, in this single-blind, placebo-controlled, diet-controlled linear study, which consisted of a 3-week placebo period, a 1-week adaptation period, and a three-week test substance period. The test substance was konjac

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[16] The NVP was stated as "...a novel, proprietary product manufactured by a proprietary process from three complementary fibers, namely xanthan, glucomannan, and sodium alginate..." known as PGX® (Vuksan *et al.*, 2009).

[17] bw = bodyweight

glucomannan (a soluble fiber) that was provided in a divided dose 1.5 g/serving over three servings, resulting in a total dose of 4.5 g/day konjac glucomannan (KGM, at approximately 82 mg/kg body weight/day). The gastrointestinal response was evaluated to determine tolerance of the test substance, and included ease of bowel movement, feeling of complete relief, abdominal cramping, borborygmi,[18] bloating, flatulence, and stool consistency. The mean frequency of defecation was significantly raised at the second and third weeks of konjac administration, compared to the third week of placebo administration. Konjac significantly decreased the difficulty of bowel movement, the incidence of borborygmi/day, and flatulence/day; compared to the third week of placebo treatment ($P<0.05$). Konjac also improved the colonic ecology, based on the types and concentrations of bacteria in the fecal contents. The authors concluded that "this study demonstrated two beneficial aspects for adding KGM (4.5 g/day) into a low-fiber diet in constipated adults. Firstly, this dose of KGM powder was well tolerated and increased stool frequency in six of the seven participants by at least 1 stool/week. Secondly, KGM improved colonic ecology by decreasing fecal pH, increasing the relative proportions of bifidobacteria and lactobacilli, and reducing the proportion of clostridia in feces" (Chen *et al.*, 2008). Vasques *et al.* (2008) administered konjac glucomannan[19] at 1.5 g/day (0.5 g before each meal)[20] over a 12-week period in a randomized, double-blind clinical study comprised of a treatment group (n = 32) and a placebo group (n = 26).[21] The subjects' ages ranged between 25 and 60, and the criteria for the subjects included having a basal metabolic index (BMI) of 30.0-39.9 kg/m^2, with stable eating habits and stable weight over the past three months, no use of drugs which might significantly affect weight, appetite, lipid profile or blood glucose levels over the eight weeks prior to the start of the study, and a good general state of health. Mean food intake and exercise levels did not change throughout the study for either group. Consumption of konjac had no effect on body mass index, body mass, percentage fat mass, or resting energy expenditure measurements ($P>0.05$). However, the treatment group had significantly decreased total cholesterol and LDL-cholesterol levels, compared with the placebo group ($P<0.05$). Adverse

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[18] Audible rumbling abdominal sounds due to gas gurgling with liquid as it passes through the intestines; http://www.iffgd.org/site/learning-center/glossary#B; site visited September 30, 2009.
[19] Konjac was administered in conjunction with a *Garcinia cambogia* extract (2.4 g/day).
[20] This amount was equivalent to 17 mg/kg body weight/day, as the subject's bodyweight averaged 90 kg.
[21] No information on the composition of the placebo was provided.

symptoms reported throughout the study (*e.g.*, abdominal pain, constipation, bloating, nausea, diuresis, gastric acidity, headache) were not significantly different between treatment and placebo groups; however, the authors stated that "of the 82 subjects initially selected, 21 dropped out during treatment and 3 were withdrawn due to significant changes in eating habits (variation of over 15% with respect to pre-treatment data). The authors did not provide any reason why 21 subjects dropped out during treatment. The results of this study must be analyzed with caution.

5. EVALUATION

PGX® is a new and unique chemical entity produced from konjac powder, sodium alginate, and xanthan gum. The intended use of PGX® as a source of dietary fiber in various products and as a dietary supplement has previously been determined GRAS. The manufacturing process has not been altered from the original GRAS determination. The consumption of PGX® when added to food products may be up to 10,070 mg/day at the 90th percentile of intake. PGX® is also contained in dietary supplements for consumption at up to 3000 mg PGX®/day. This GRAS amendment includes an evaluation of the consumption of PGX® as an ingredient in medical foods, either as a sole source of the diet or as part of a balanced diet that includes conventional foods. Medical foods are consumed under a physician's care with ongoing medical supervision, and based on clinical practice, the physician should determine the sources of fiber in the diet, including the use of dietary supplements, and would provide medical foods containing PGX® to those individuals with inadequate fiber intake from conventional foods. Therefore, it is likely that the dietary intake of PGX® will not exceed 13,070 mg/day at the 90th percentile, consistent with the safe use of PGX® as determined in the original GRAS determination. Consumption of PGX® from food, dietary supplements, and medical foods is not expected, but would result in a 90th percentile consumption at 13,070 mg/day. Consumption of various forms of fiber at 32 – 40 g/day for up to three weeks resulted in flatulence and bloating, but no serious adverse effects. Consumption of PGX® at up to 16 g/day is not expected to result in serious adverse effects, and would be self-limiting.

For completeness sake, a review of the literature on the components of PGX® was included in this amendment. A short-term study in dogs that received up to 2000 mg/kg body weight/day of xanthan gum for ten days did not result in adverse effects. Konjac administered at

approximately 7500 mg/kg body weight/day in the diet to mice was well tolerated, with no reported adverse effects. Sodium alginate provided to rats in the drinking water at approximately 0.7 mg/kg bw/day for three weeks was reported not to induce adverse effects, but did decrease lesion formation in TNBS-induced colitis. PGX® was found to be nongenotoxic in a bacterial reverse mutation assay and a mammalian erythrocyte micronucleus assay at any of the PGX® levels evaluated.

A clinical trial with a single administration of PGX® at 5.1 g (approximately 85 mg/kg bw/day for a 60 kg person) did not increase incidences of any adverse effects. Konjac consumed at up to 4.5 g/day for three weeks by constipated female subjects decreased the difficulty of bowel movement, borborygmi/day and flatulence/day, and konjac consumed at 17 mg/kg body weight/day for twelve weeks did not adversely affect body mass parameters. The preclinical and clinical studies further support the safety and the GRAS status of the intended uses of PGX®.

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6. CERTIFICATION

The undersigned authors of this document—an amendment to the dossier in support of the GRAS status determination of PGX®, for use of PGX® in medical foods—hereby certify that, to the best of their knowledge and belief, this document is a complete and scientifically balanced representation of all available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

15OCT09

Ray A. Matulka, Ph.D.
Director of Toxicology,
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Date

15OCT2009

George A. Burdock, Ph.D.
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Date

7. CONCLUSION

We, the Expert Panel, have individually and collectively critically evaluated information generated since the first GRAS review summarized in this amendment for the GRAS dossier (Dossier in support of the Generally Recognized As Safe (GRAS) status of PolyGlycopleX® (PGX®) as a food ingredient). This information is consistent with that provided in the initial GRAS determination and fully supports the safety of the new intended uses of PGX® as a source of dietary fiber in medical foods at an aggregate daily intake of up to 13,070 mg, as monitored by the health care provider. We further conclude that the new intended use of PGX® as an ingredient when added to "medical foods" is GRAS based on scientific procedures and therefore is consistent with the earlier GRAS determination.

It is our opinion that other experts qualified by scientific training and experience to evaluate the safety of food and food ingredients would concur with these conclusions.

8. SIGNATURES

22 October 2009
Date

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20 Oct 09
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Submission End

Reference List for Industry Submission, GRN 000328

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NA- Not applicable